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Multiagent-Based Simulation of the Human Immune System: A Study of the Immune Response and Antimicrobial Therapy in Post-Streptococcal Glomerulonephritis

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Abstract

The study of the human immune system is an important research area with results that can help improve public health. The human immune system is a natural multiagent system. It is, therefore, liable to be simulated by a corresponding artificial multiagent system. The purpose of this study is to propose a multiagent system for conducting experiments that contribute to highlighting the role of humoral immunity in post-streptococcal glomerulonephritis (PSGN). We identified the requirements for extending the AutoSimmune simulator to simulate key phenomena involved in the emergence of PSGN. Two environments were included: kidney and upper respiratory tract. We also included simulation of neutrophil cells. Furthermore, we simulated the administration of antimicrobials, reporting the consequent reduction of PSGN risk.

Keywords: Computer simulation; immune system; post-streptococcal glomerulonephritis; *Streptococcus pyogenes*; multiagent systems

Introduction

The human immune system (IS) is highly complex and is considered one of the most challenging topics of biology (Possi et al. 2011) (Folcik, An, and Orosz 2007). Nevertheless, knowledge about its mechanisms is fundamental to the advancement of several areas of science, including medicine and computer science, which makes this biological system a mandatory research topic. Given its importance, several *in vivo* and *in vitro* methods, as well as mathematical models, have been developed to study the immune system. Additionally, due to its high complexity, there is growing interest in creating computational models, called *in silico* models, to be used in conjunction with more traditional approaches of medical research (Folcik, An, and Orosz 2007).

Multiagent system (MAS) modeling approaches have been successfully applied to simulate many complex systems. A MAS-based method allows the exploration of macroscopic behavior emerging from microscopic interactions. For this reason, MAS is considered by many authors the best approach to model the immune system (Folcik, An, and Orosz 2007) (hua Li et al. 2009) (Holcombe et al. 2012). Its main disadvantage, however, is the high computational cost when a large number of agents is used (hua Li et al. 2009). It is worth noting that, when this approach is applied to system modeling, one has to define which elements of the domain will be represented by agents, i.e., the level of granularity, and then establish their behavior rules. As a result, the system's behavior emerges from interactions among agents as well as between agents and the environment. By defining these agents, we cannot try to represent all types of entities, since the computational complexity grows exponentially with the number of agents (hua Li et al. 2009). Instead, we focus our representation on what has to be modeled, obtaining as coarse as possible granularity, but still preserving the entities and mechanisms required to simulate the behavior we intend to study.

An important application of MAS-based immune system simulation is the study and testing of hypotheses about a disease. One such disease that has a large social impact is post-streptococcal glomerulonephritis (PSGN). PSGN is an acute inflammatory disease involving renal glomeruli. Pathologically, it is presented as diffuse proliferative lesions and arises in one to four weeks after a skin infection or an upper respiratory airways infection by *Streptococcus pyogenes* (Kumar et al. 2009). The disease is characterized by hematuria, edema, as well as hypertension, and it may progress to acute renal failure. Nephritic syndrome by PSGN usually affects six-to ten-year-old children (Kumar et al. 2009), (Bisno 2000). PSGN's epidemiological pattern has undergone important changes in the last three decades, becoming rare in developed nations. However, PSGN is still occurring in developing countries, where the incidence is between 9.5 to 28.5 new cases per 100,000 individuals per year (Rodriguez-Iturbe and Musser 2008). According to (Rodriguez-Iturbe and Musser 2008), about 456,000 PSGN cases per year occur in developing countries out of 472,000 cases worldwide.

The aim of this research is to carry out an *in silico* analysis to corroborate the role of humoral immunity in PSGN and to investigate the evolution of pathophysiological processes in this condition when antimicrobials are administered. This was accomplished by extending the simulator

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AutoSimmune (Possi et al. 2011). An initial proposal of this work was previously published in (Bastos et al. 2013), but in that article was not addressed the evolution of pathophysiological processes when antimicrobials are administered. This paper is structured as follows: The next section describes the requirements for the simulation of PSGN; the section entitled *The Model* describes the general characteristics of the simulator; the subsection entitled *Evidence of deposit of immune complexes in renal glomeruli characterizing PSGN* presents the simulation results showing the characterization of PSGN by deposition of immune complexes in renal glomeruli; the subsection entitled *Administration of antimicrobials and reduced risk of PSGN occurrence* presents the simulation results showing the reduced risk of PSGN due to antimicrobial administration; finally, the *Conclusion* section presents the study's conclusions.

Methods

In order to perform the simulation related to the emergence of PSGN, first the requirements for this simulation had to be captured and, next, the required entities were modeled. These steps are detailed below.

Requirements for PSGN Simulation

To study the role of humoral immunity in PSGN, the mechanisms related to the causes of the disease must be simulated. In addition, it should be emphasized that the disease manifests as an immune complex-mediated reaction. Many cationic antigens, such as NAPlr, SPEB, and zSpeB are suspected of being involved in nephrotogenicity (Bisno 2000). Laboratory tests detect low complement levels and higher anti-Streptococcus (anti-streptolysin O), which confirms the previous infection by this pathogen.

According to (Kumar et al. 2009), the relationship of the disease with the formation of immune complexes is confirmed through optical microscopy by the presence of granular immune deposits. The glomerulus is augmented due to hypercellularity, which is caused by proliferation of endothelial and mesangial cells. In severe cases, crescents are formed. The requirements identified as significant for computational modeling were as follows:

1. Simulation of a *Streptococcus pyogenes* infection in the upper respiratory tract or the skin. After this infection, *S. pyogenes* antigens are released.
2. The affinity between the pathogen and host tissue to which the pathogen adhere and infect, starting the proliferation process, should be simulated. Once infection is established, adaptive immunity comes into play, primarily humoral adaptive immunity mediated by antibodies secreted by B lymphocytes. These antibodies bind to the *S. pyogenes* antigens in order to neutralize and eliminate these microorganisms. From this point onwards, antigen-antibody complexes (immune complexes) are formed, which flow through the bloodstream and are filtered in the kidney.
3. The deposition of immune complexes causes an inflammatory reaction in the kidney. The model should be able to simulate the deposition of immune complexes, as well as the infiltration of cells (neutrophils and macrophages, among others) occurring in glomeruli or renal tissue.
4. Another requirement detected was the inclusion of an agent to represent the behavior of the antimicrobials that are capable of controlling bacteria multiplication in the host. The goal is to verify whether antimicrobial administration in the early stage of infection is able to prevent the release of antigens that cause the PSGN pathology.

The Model

The model was developed focusing on its implementation in the Repast Simphony framework[1], version 1.2. Possi (Possi 2012) developed the initial version of the simulator and set the representation of several important elements for immune system simulation such as space, time, and representations of cells, tissues, and substances, resulting in an immune system simulator called *AutoSimmune*. Since then, the simulator has been applied to test hypotheses on autoimmune diseases (Possi et al. 2011) and on the behavior of immune system cells (Da Silva et al. 2012). This paper describes extensions relevant to the study of PSGN. For clarity, it briefly reports the characteristics of AutoSimmune.

AutoSimmune Simulator AutoSimmune is an immune system simulator with original focus in autoimmunity. In its basic version, it simulates bone marrow, thymus, lymph nodes, blood circulation, and parenchymal tissue region. The regions are simulated as a discrete space in a two-dimensional grid in which each agent has a position (*i,j*). More than one agent can occupy the same position, which somehow simulates a 3D space. The agent is moved by changing its position to a new position in the *Moore neighborhood* (Weisstein 2005). Thus, an agent cannot skip positions, i.e., it needs to move one position at a time. In such two-dimensional-grid structure, the Moore neighborhood (of radius one) comprises the eight neighboring position to a central position. If allowed in its specification, an agent can move from one region to another by means of special elements called portals, as proposed in (Folcik, An, and Orosz 2007). Substances such as cytokines are simulated by means of layers of data provided by the Repast framework, called *ValueLayer*. As substances are released by cells, they undergo a process of diffusion, spreading into the surroundings of the site where they were released, thus decreasing in concentration, and also undergo a process of decay, decreasing in amount over time (Possi 2012). *ValueLayer* is an abstract layer of data that, at the time of its creation, is associated with a region of the grid. Multiple layers of data can be combined at the same grid. Thus, an agent can know the concentration of a given substance at that time instant at position (*i,j*).

The passage of time is modeled using the concept of discrete time unit called *tick* provided by the framework. Each agent defines when to start to be called and the interval of each call. Ticks are the time intervals necessary for the transition from a state of the environment to the next. Therefore,

[1] http://repast.sourceforge.net/

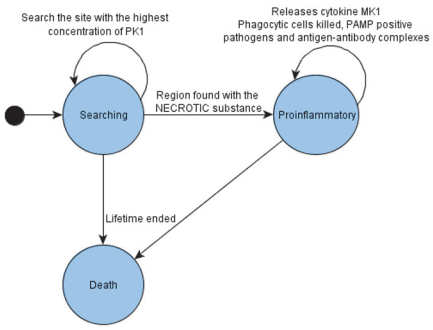

Figure 1: Neutrophil agent states (Bastos et al. 2013).

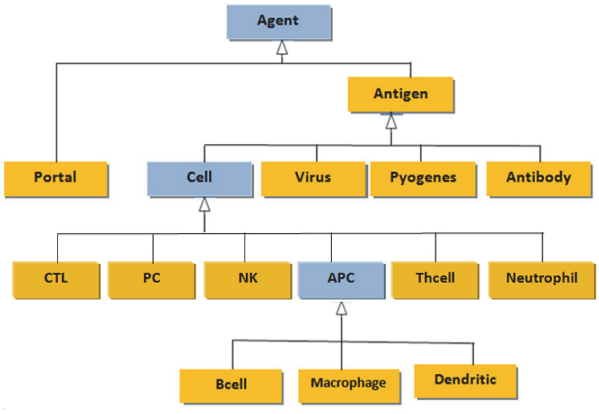

Figure 2: Agent classes and their hierarchical organization.

all events scheduled to be executed must be completed before the next round occurs. Thus, during a tick, all agents scheduled for the given time will change their positions, release a substance, and analyze their neighborhoods based on information from the previous tick. Only when every agent has made its actions does the tick end and the information is updated (Possi 2012).

In the simulator, the affinity (which is the recognition strength of an antigen by a receptor) is simulated by the number of matching bits between two bit sequences: One belonging to a cell receptor and another belonging to the antigen. The greater the length of the match, the greater the affinity. To calculate the affinity, we used the method suggested by (Floreano and Mattiussi 2008), called the "length of the longest common subsequence," whose goal is to compute, given two patterns of bit sequences A and B, the size of the largest contiguous subsequence of bits contained in A and B simultaneously, in the same order.

Extensions to simulate PSGN The standard version of AutoSimmune features several agents relating to immune cells such as NK, cytotoxic lymphocytes, B lymphocytes, macrophages, and dendritic cells. In addition, the system provides agents that mimic entities such as viruses, tissue cells, antibodies, and portals (to allow the passage of an agent from one region to another). For the simulation of PSGN, agents to simulate *S. pyogenes*, neutrophils, and immune complexes had to be added. Regions also had to be added to simulate the respiratory tract (*AirWayTissue*) and renal tissue (*KidneyTissue*). The agents were modeled using state diagrams. Due to the lack of space, only the state diagram of the agent that simulates the neutrophil is shown here in Figure 1. The state diagram of each agent is deterministic, but the interaction between agents is nondeterministic, which results in a nondeterministic simulation.

According to (Abbas, Lichtman, and Pillai 2010), neutrophils respond to most infections, particularly the events caused by bacteria and fungi. Their main roles are phagocyting microorganisms, removing tissue debris, and acting in the extra- and intracellular environments to lyse and degrade microorganisms through the digestive enzymes present in their cytoplasmic granules while adaptive immunity is preparing to act. Phagocytized microorganisms, coated with complementary and specific antibody (opsonization) are killed by a combination of toxic oxygen radicals produced by neutrophils and cytotoxic proteins derived from the cytoplasmic granules.

The criteria to define what would be modeled as agents were the same adopted by Possi (Possi 2012), i.e., everything that is included in the model and is an antigen, or contains antigens, is modeled as an agent. In other words, any component that can be recognized by a PRR (Pattern Recognition Receptor) was considered an agent. Figure 2 shows the classes representing which elements were modeled as agents. In blue are the abstract classes, and in yellow, the concrete classes.

Results

Evidence of deposit of immune complexes in renal glomeruli characterizing PSGN

According to (Eison et al. 2011), immune deposits on electron-mesangium and/or along the basement membrane can be observed through electron microscopy in the occurrence of PSGN. Once deposited in the kidney, immune complexes can be degraded mainly by infiltrating neutrophils and monocytes/macrophages, mesangial cells, and endogenous proteases, resulting in remitting of inflammatory reaction. PSGN is an immune-mediated disease (Kumar et al. 2009). The latency period between infection and the onset of nephritis is compatible with the time needed to produce antibodies against *Streptococcus* antigens, which are present in most patients.

The first step needed to start simulations was identifying the number of bacteria which would be required. We used different configurations of the system parameters to initiate the inflammatory process in the *KidneyTissue* (kidneys) cells. After the system became stable, simulations were performed using the following parameters: **Initial number of Pyogenes**, representing how many bacteria would start the simulation; **Pyogenes Virulency**, representing the ability of the bacteria to reproduce, or how many times it can reproduce; and **Pyogenes Latency**, representing for how many *ticks* the organism will survive. Seventeen experiments were

conducted with different variations of these parameters in order to evaluate the behavior of zones *AirWayTissue* and *KidneyTissue*. The goal was to select the inoculum with the fewest bacteria that would produce an inflammatory reaction in *KidneyTissue*. After the tests, it was verified that the minimum values for the parameters resulting in inflammation were: **Initial number of Pyogenes** = 300; **Pyogenes Virulency** = 2; **Pyogenes Latency** = 10. We simulated an infection in airways (*AirWayTissue*) occurring after infiltration of neutrophils and macrophages to the sites where there is deposition of antigen-antibody complex (*Zone KidneyTissue* in the model, representing kidneys).

Figure 3 shows a screen of the AutoSimmune simulator in operation. On the left are the template settings, which are set before the start of the simulation. The several zones can be accessed through the tabs on the bottom right of the screen. The zone displayed is the *AirWayTissue*. The grid-shaped structure that appears in the zone is the structural organization of a hypothetical tissue. We can see from this figure that an infection has been started in the simulation.

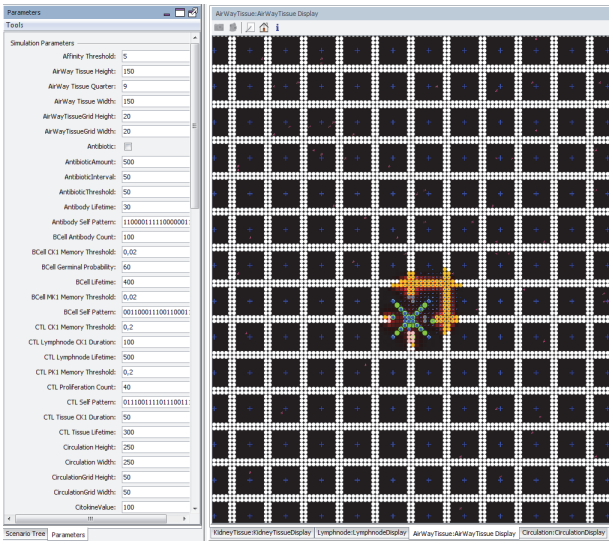

Figure 3: Initial screen of AutoSimmune.

Figure 4 shows details of the zone *AirWayTissue*: Parenchyma cells (PC), shown as white circles, are being infected by the agent pyogenes (*Streptococcus pyogenes* bacteria, represented in blue). Also in blue, macrophages initiate an inflammatory response along with neutrophils (white circles). Red circles represent antibodies, while blue crosses represent portals. The red background represents the concentration gradient of the substance PK1, which indicates the stress state of parenchymal cells. PCs come in a few different colors: white (normal), yellow (stressed), and gray (infected, dying). Additionally, antibodies are illustrated by red circles, neutrophils are represented by green circles, macrophages are indicated as rounded blue shapes, and pyogenes, seen in greater number, are shown as small blue shapes.

Figure 5 shows the evolution of the infection with the

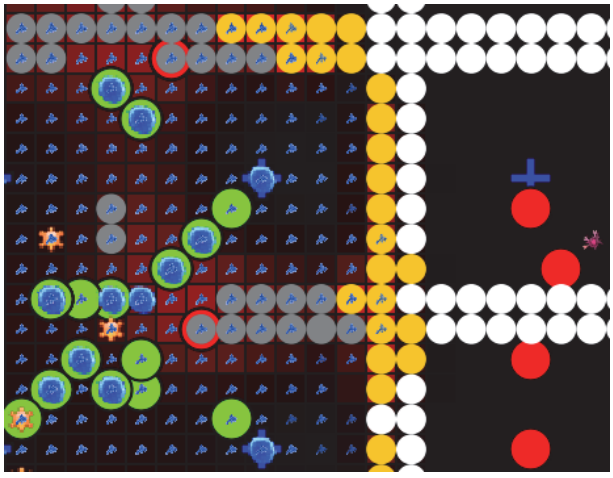

Figure 4: AirWayTissue - Enlarged view of the moment of infection by *Streptococcus pyogenes* bacteria (in blue, inside several tissue cells)

number of agents in the simulation at each time instant. It also shows the moment when bacteria are detected in the tissue *AirWayTissue*. After the entry of bacteria, the innate immune system starts to act immediately. NK cells, macrophages, and neutrophils begin to engulf bacteria as well as necrotic cells and begin to send signals to other inflammatory agents. The adaptive response is initiated and dendritic cells activate B cells that produce antibodies. These antibodies flow through the bloodstream and, according to their specificity, bind to antigens from pathogenic bacteria. As they pass through kidney filters, immune complexes are trapped in glomeruli. Then, another form of inflammatory response is initiated. Neutrophils and macrophages are directed towards where the immune complexes were fixed. As a result, the process of glomerulus injury is started, causing glomerulonephritis.

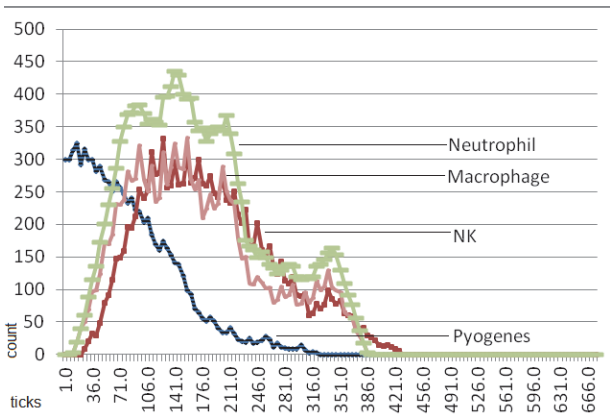

Figure 5: Evolution of infection in the *AirWayTissue*.

Figure 6 illustrates the evolution of the infection with the participation of adaptive immunity in tissue *AirWayTissue*. The main property of adaptive immunity is its specificity,

i.e., only cells that recognize the antigen of *S. pyogenes* proliferate. A large amount of specific antibodies that bind to antigens are produced and transported by blood circulation and are filtered by the glomeruli, where they attach.

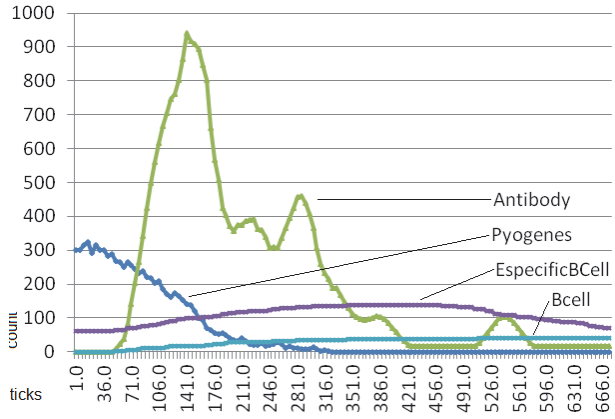

Figure 6: Evolution of infection with the participation of adaptive immunity in the *AirWayTissue*.

Figure 7 illustrates the evolution of the inflammatory process in kidney cells (*KidneyTissue*), expressing the time at which deposition of immune complexes occurs on kidneys cells, with recruitment of neutrophils, macrophages, and NK cells to the site of attachment, creating an inflammatory process, characteristic of PSGN. As seen, it is an essential scenario for the occurrence of the pathological condition where immune complexes attach to the glomeruli. This is only possible when there is a humoral immune response mediated by secreted antibodies, whose main physiological role is to respond to extracellular microorganisms and microbial toxins (Abbas, Lichtman, and Pillai 2010). Thus, the simulation confirmed the hypothesis on the role of humoral immunity in this disease.

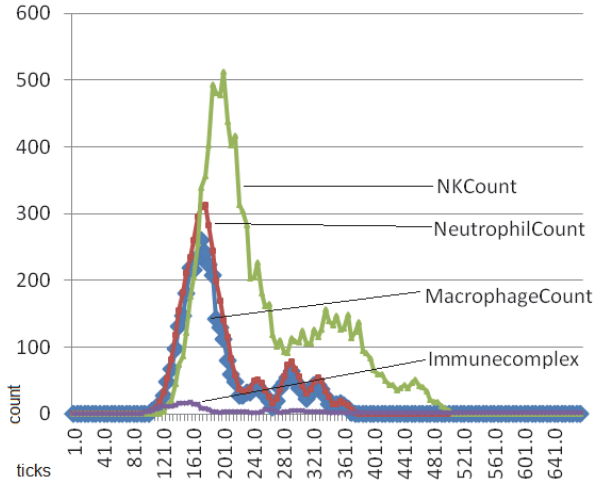

Figure 7: The inflammatory process occurring in kidney glomeruli.

Administration of antimicrobials and reduced risk of PSGN occurrence

The purpose of this experiment is to show the behavior of the model when antimicrobials are administered, such as antibiotics. The mechanism of action of many antimicrobial drugs is not fully elucidated. However, such mechanisms are generally divided into the following categories (Siqueira-Batista et al. 2011): (1) inhibition of cell-wall synthesis, (2) inhibition of cell-membrane function, (3) inhibition of protein synthesis, and (4) inhibition of nucleic-acid synthesis.

We did not specify the mechanism of action of the antimicrobial. However, we defined the following parameters: (1) amount of antimicrobial to be released in the tissue (*Antibiotic Amount*); (2) frequency of release (*Antibiotic Interval*); (3) level of penetration into bacteria (bacterial resistance) (*Antibiotic Threshold*).

In our experiments, we used the previously mentioned parameters since they were identified as the minimum enough to cause PSGN. Several experiments were performed combining the three new parameters mentioned above in order to identify values that indicate the efficiency in response to bacterial infection, noticing whether or not the development of PSGN would occur in this context. The experiments revealed that the best combination of parameters for the use of antimicrobials in order to avoid the occurrence of PSGN were: **Number of ticks**= 401, **Initial number of Pyogenes**= 300; **Pyogenes Virulency**= 2; **Pyogenes Latency**= 10, **Antibiotic Amount** =500, **Antibiotic Interval**= 10 and **Antibiotic Threshold**= 15. Figure 8 shows the rapid reduction in the number of *Pyogene* agents in the *AirwayTissue* due to the antimicrobial application.

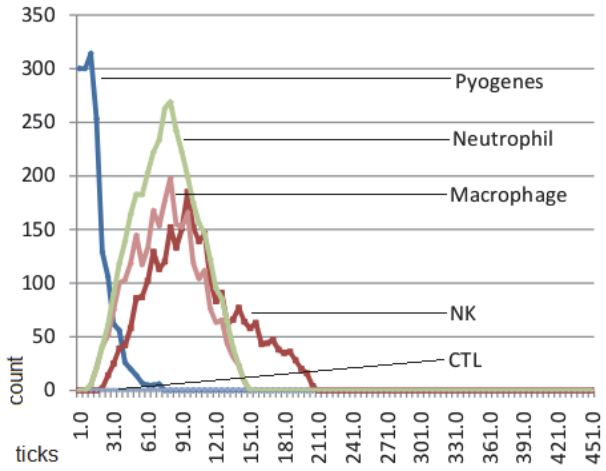

Figure 8: *AirWayTissue* - with antimicrobial application.

In this experiment, the number of immune complexes, neutrophils, macrophages, and NK cells remained equal to zero in the area of *kidney tissue*. Therefore, no PSGN occurred due to the application of antimicrobials in the *AirWayTissue*.

There was no retention of immune complexes and, consequently, there was no inflammatory reaction. As a result,

PSGN has not been established. We conducted another experiment, increasing the number of bacteria fivefold, starting with 1,500 bacteria. Figure 9 shows that, even with a larger bacterial load, antimicrobials prevent the occurrence of PSGN. We concluded that the use of antibiotics can minimize the occurrence of PSGN. Moreover, experiments carried out in the simulations represent a tool that could allow the role of antibiotics in infectious processes to be investigated in future researches of the group.

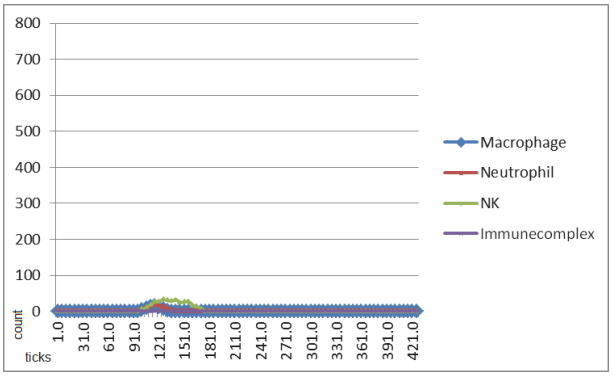

Figure 9: *KidneyTissue* simulation with 1,500 bacteria and antimicrobial application.

Conclusion

In this study, we posed the requirements for a model to assist in the study of the processes involved in PSGN. The extended simulator was able to exhibit behavior consistent with expectations, according to the literature. An important result is the corroboration of the importance of antimicrobial administration to prevent the occurrence of PSGN. The results obtained by the simulation model are not to be interpreted as definitive evidence of the pathophysiological events in PSGN. However, the data offer biological and clinical evidence which highlights the importance of further investigation on PSGN. Furthermore, we could deliver a powerful computational framework for *in silico* hypothesis testing for advanced studies of this important disease.

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