

Plasmodium falciparum INFECTION*

IN SILICO PRELIMINARY STUDIES

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Resumo

A malária é uma doença infecciosa de grande impacto em termos de saúde pública – dado o contingente de pessoas afetadas e submetidas ao risco de adoecer –, causada por protozoários do gênero *Plasmodium*. São conhecidas cinco principais espécies capazes de infectar humanos: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* e *Plasmodium knowlesi*, destacando-se a primeira como aquela capaz de produzir os quadros de maior gravidade. Apesar de sua relevância clínica e epidemiológica e das investigações em desenvolvimento – dirigidas aos diferentes aspectos da interação entre o homem e os protozoários do gênero *Plasmodium* – permanecem inúmeras dúvidas sobre distintos aspectos do processo fisiopatológico da malária. Para estudar tais lacunas, pode-se buscar estratégias interdisciplinares envolvendo biologia, medicina e ciência da computação, no âmbito da experimentação *in silico*. Tal abordagem apresenta rapidez, baixo custo e a não implicação em questões éticas que permeiam as experimentações *in vitro* e *in vivo*. Com base nessas considerações, o presente artigo apresenta os resultados preliminares de um modelo computacional de interação entre *P. falciparum* e eritrócitos, os quais foram implementados – computacionalmente – no sistema *AutoSimmune*. Os resultados obtidos demonstram que o sistema é capaz de simular o processo de infecção das células hospedeiras pelo protozoário, apresentando, assim, similaridade com a realidade biológica.

Palavras-chave: Ciclo eritrocítico. Malária. Medicina Computacional. *Plasmodium falciparum*.

Abstract

Malaria is an infectious disease of great impact in terms of public health, given the number of people affected and subjected to the risk of illness. Protozoa of the genus *Plasmodium* cause it and five species can infect humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi*; the first is able to produce the most severe cases of the disease. Despite its clinical and epidemiological relevance and investigations in development – targeted at different aspects of the interaction between humans and *Plasmodium* protozoa of the genus – there remains many questions about different aspects of the malaria pathophysiology. To study such gaps, interdisciplinary strategies can be pursued, which involve biology, medicine and computer science, as part of the trial *in silico*. Such approach provides agility, low cost and does not imply ethical issues that permeate the experiments *in vitro* and *in vivo*. Based on these considerations, this article presents preliminary results of a computational model of the interaction between *P. falciparum* and erythrocytes, implemented in *AutoSimmune* system. The results obtained show that the system is able to simulate the host cells infection process by protozoan with similarities with the biological reality.

Keywords: Erythrocytic cycle. Malaria. Computational Medicine. *Plasmodium falciparum*.

1 INTRODUCTION

Malaria is a parasitic disease caused by the protozoa *Plasmodium*, highlighting five species of parasites that can infect humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi*. The first two agents are the most important; *P. vivax* is responsible for most cases in Brazil, however *P. falciparum* is responsible for the most severe clinical conditions, which evolve more often to complications that can increase the mortality (WORLD HEALTH ORGANIZATION, 2014). The disease is transmitted to humans by the *Anopheles* mosquitoes female. When feeding, usually at dusk and /or dawn, these insects inoculate the parasite in the host, causing the illness, with subsequent clinical malaria and its complications - particularly the injuries of the nervous system, severe anemia, acute renal failure, shock, the pulmonary and hepatic dysfunction, disseminated intravascular coagulation and hypoglycemia (GOMES et al., 2011; WORLD HEALTH ORGANIZATION, 2014).

The disease is incident in many countries in sub-Saharan Africa, with characteristics of a public health problem of world order. Malaria also has a significant impact in terms of public health – in fact, is the worldwide largest impact disease among parasitic – and it is estimated that about half the world population is at risk of developing the disease (WORLD HEALTH ORGANIZATION, 2014). In this context, in 2012, malaria was responsible for the involvement of 207 million people worldwide, with approximately 627,000 deaths, affecting mostly children under five years old (about 482,000 children). Since 2000, with increased coverage of prevention and control programs, there was a reduction of 42% of global mortality. This reality has been considered a significant step forward, demonstrating the ability to control this protozoan, when applied technical resources and adequate financial programs (WORLD HEALTH ORGANIZATION, 2014).

The Brazilian reality can be observed when we analyze the last 14 years (2000-2013), which have recorded, according to the Brazilian Ministry of Health, an average of 392,600 annual cases of malaria in the country. Currently, these numbers are decreasing and the amount of cases of the disease recorded in recent years went down: 267 047 cases in 2011, 242,756 in 2012 (a reduction of 9.1%) and 178,613 in 2013 (down 26.4%). The predominant geographical location of cases is in Legal Amazonia (99.5%), with preponderance in the states of Acre, Amazonas, Pará and Rondônia (PINA-COSTA et al., 2014).

Although the scientific community has made important advances in the understanding of the pathophysiology, diagnosis and treatment of malaria, the disease still keeps up with high fatality rates (especially in the case of *P. falciparum* infection), more pronounced in tropical developing countries (BUCHALLA et al., 2003; LUNA, 2002; WORLD HEALTH ORGANIZATION GLOBAL MALARIA PROGRAMME, 2013). The need to take measures of control and prevention is an important issue and essential for improving the quality of life of the populations affected by the endemy. Given this reality, several investigations are under development, aimed at different aspects of the interaction between man and the genus *Plasmodium* proto-

zoa. In this context, works related to immunological studies are gaining ground, especially those with the objective of developing immunization to malaria, since turning it into a vaccine-preventable disease would bring great benefit to the world community. However, the success in terms of production of a vaccine for use in the general population has not been fully achieved. There are serious obstacles to vaccine development, among which stands out the technical complexity, due to the various developmental forms in the biological cycle of the parasite (CDC, 2016). However, according to WORLD HEALTH ORGANIZATION (2014), although there is still no malaria vaccine, many studies have been made; the one with the greatest advances for *Plasmodium falciparum* is the RTS S / AS01. These results are being analyzed by a significant clinical trial with 15,460 children, in seven countries in the sub-Saharan Africa, and has shown promising results (WORLD HEALTH ORGANIZATION GLOBAL MALARIA PROGRAMME, 2013).

Plasmodium presents different evolutionary forms (BUCHALLA et al., 2003), leading to the involvement of different antigens at various stages of its life cycle (SIQUEIRA-BATISTA et al., 2012a). Furthermore, this protozoon has the ability to modify the molecules of their antigens (antigenic variation) on a mechanism to “escape” aiding common to other species of organisms that infect humans (FAIRHURST; WELLEMS, 2015). The protists use also many other mechanisms to escape from the protective immune response of the host, like proliferation within the cells, which makes difficult the access, for antibodies from the host (ABBAS et al., 2008).

Vaccine development efforts have been done, using algorithms that search for immunogenic epitopes. It is unclear whether this empirical approach can be applied to find antigenic determinants associated with protective immunity to new antigens. The limited precision of the algorithms predicts tested for immune responses *in vivo* emphasizes the need to improve the predictive capabilities to use them as tools in the development of a vaccine (BERGMANN-LEITNER et al., 2013).

Given the difficulties mentioned prior in the text, there has been sought new ways to investigate this important disease, neglected in the globalized world. The proposal has been the “alliance” between biology, medicine and computer science, which allows the development of experiments *in silico*, which is promising on malaria research (DINGA et al., 2014).

Thus, from these considerations, the aim of this article is to (1) review aspects of the etiological agent of the biology of malaria and computer modeling of the human immune system using multi-agent systems and to (2) present, in a preliminary way, computer simulation aspects of the interaction between *Plasmodium* / *Homo sapiens sapiens* using *AutoSimune* system.

2 LITERATURE REVIEW

2.1 Biological Aspects

Plasmodium protozoan – due to the apical complex of specialized organelles (dense granules, and micronemes rhoptries), involved in invasion of host cells – belongs to the phylum *Apicomplexa* (FAIRHURST; WELLEMS, 2015). The protist has several evolutionary forms:

(1) Sporozoites – Inside it there are cellular structures, including a core and mitochondria (GARCIA et al., 2006). After the penetration into a hepatocyte, each sporozoite becomes cryptozoites and the core enters in a rapid multiplication process forming. Within three days, thousands of hepatic merozoites disrupt liver cells, enter the bloodstream and so enter the red blood cells.

(2) Liver merozoites – which are structurally similar to the sporozoites, however, they are smaller and more ovoid than these, with a structure that allows the invasion of erythrocytes and start the erythrocytic cycle. After this, it becomes a trophozoite (INGMUNDSON et al., 2014). They move through pseudopodia and have no unique morphology. Due to its high synthesizing capacity, the amount of core is high and these begin to divide; the membrane is doubled, the apical complex is produced and fingerings are formed in the cell membrane and cytoplasm. The trophozoite is called in this moment schizonte and it reproduce asexually, producing thousands of merozoites, that can infect new red blood cells, again.

(3) Blood merozoites – these come from rosacea fragmentation, with smaller dimensions than the liver merozoites, and have the ability to enter red blood cells (STANISIC et al., 2015).

(4) Gametocytes – these are formed from blood trophozoites. The female gametocytes, known as macrogametocytes, are more abundant in the blood stream, they have rounded form and fill almost all the red blood cell, excluding *P. falciparum*, which present – structurally – a banana shape, they cause deformation and / or breakage on erythrocyte (MORAHAN; GARCIA-BUSTOS, 2014). Male gametocytes, the microgametocytes, become circular and bring up flagellar extensions provided with a core, which are the forms sucked by the *Anopheles* mosquito. After leaving the main cell, they become the male gametes, provided by accelerated vibratory motion of structures. This process is known as exflagellation.

(5) Ookinete – these are formed in the stomach of the *Anopheles* spp., from the merger of microgamete and macrogamete. The latter results from changes in macrogametocytes. When it allocates in the stomach epithelium of the *Anopheles*, it organizes a protective capsule and is named oocysts, which later will give rise to the sporozoites, restarting the cycle (VEGA-RODRÍGUEZ et al., 2014).

In the case of infections by *P. vivax*, relapse can occur after a few months of the first malaria episode, and even after a successful treatment. This is explained by the occurrence of pre-erythrocytic cycles and later erythrocytic originated from the sporozoites, which remained quiescent in the liver during this time. This prolonged incubation period characteristic – referred

as hypnozoite (derived from Greek *hypnos*, sleep) – is part of the gene in some species and strains of *Plasmodium* (REY, 2008).

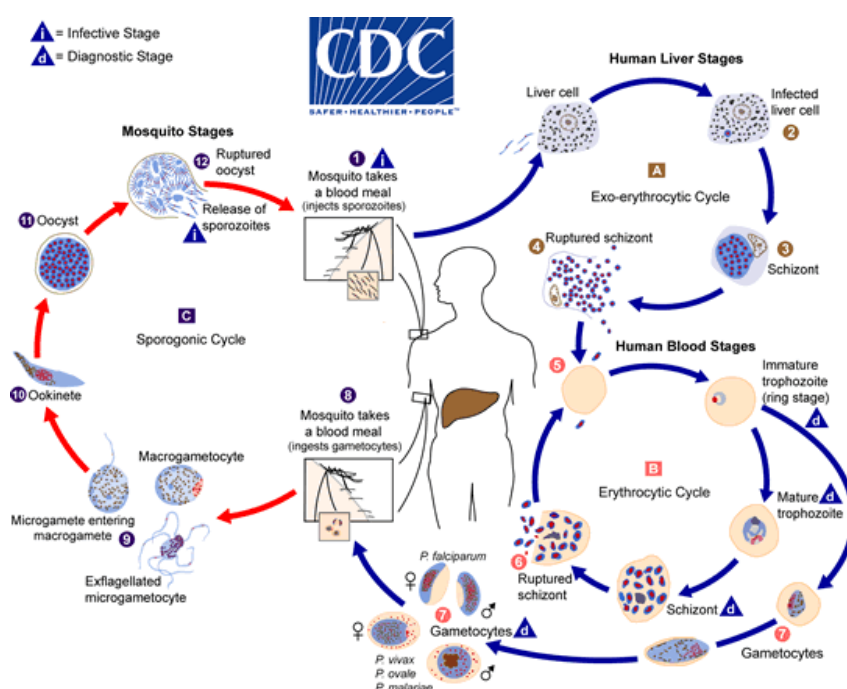
The life cycle of *Plasmodium* – in Figure 1 – is initiated through the ingestion by the insect, of human blood containing gametocytes. Such structures emerge from the red cells within the mosquito gut, as male and female gametes, and, after the union of both, they differ in oocinetos (zygote diploid), which penetrate the wall of the gastrointestinal tract into the *Anopheles*, and becomes an oocyst (which can hold up to thousand sporozoites). Subsequently, they are carried through the hemolymph, and spread to the salivary glands of the *Anopheles*, a process that can last for up to two weeks (REY, 2008; FAIRHURST; WELLEMS, 2015).

The sporozoites are inoculated in humans through the bite of the female mosquito – they spend several hours to cross the dermal tissue and cellular barrier – and reach the blood and lymphatic system, where they are taken to the liver and invade the hepatocytes. Next they differentiate in criptozoytes that differentiate into merozoites.

The hepatic cycle shown by Short and Garnham, 1948 (PERKINS, 2014), has a specific sequence: (i) contacting the host cell; (ii) signaling events with discharge of calcium; (iii) release of molecules from the apical ligands and complex – that culminates in cell invasion through a parasitophorous vacuole.

Each hepatocyte supports the development of 10000-30000 merozoites. There is no correlation between these numbers and clinical changes. This process, in the liver lasts about one or two weeks for *P. falciparum* and *P. malariae*, while for *P. vivax* and *P. ovale* may last for days or so, and it can remain in a latent form as the protozoan takes the form of hypnozoite for months or years, which is the cause of resurgence of malaria (NAGARAJ et al., 2013).

Figure 1 – Malaria Cycle



Source: Centers for Disease Control and Prevention

Available at: <<http://www.cdc.gov/malaria/about/biology>>

After the outbreak of hepatic cell, the pathogen moves – through a potent “engine” of actin and myosin – to meet the *erythrocytes* and invades it through a *parasitophorous vacuole* (MALPEDE; TOLIA, 2014; WOLDEAREGAI et al., 2013). They use the apical complex components to promote their entry; and migration to the interior – dependent on energy – starting the intra-erythrocyte cycle.

Inside the erythrocyte, the merozoites differentiate into trophozoites, and multiply by schizogony erythrocyte, becoming a formation known as rosacea or merozoite, each one of this bodies is called schizontand, after their separation and hatching of the blood cell, they differ again in merozoites which are able to re-infect red blood cells.

The aspects of this cyclic of invasion and multiplication favor the existence of a considerable parasitic biomass with exponential growth, which cause fever and other disturbance processes, such as anemia and / or occluding vascular beds (BUFFET et al., 2011).

2.2 Computacional Approach: Research *In Silico* Using Multiagent Systems

Computational technological innovations have allowed the use of *in silico* experimentation in different fields of knowledge, including the life sciences and health. The experiments *in silico* are considered fast, inexpensive and do not imply ethical issues described in experiments *in vitro* and *in vivo* (GOMES et al., 2015). This type of research is based on the initial model, devised in the light of existing knowledge and in the observation of the phenomena under study. Thus, it is possible to test several hypotheses, using a simulation tool, and the results can be contrasted with experimental observations of real phenomena. This fact allows to validate or indicate the need for issues in the model, and provides clues to be incorporated into the scientific theory about the phenomenon under study (GOMES et al., 2015).

Among the approaches described for biological studies *in silico* are the Multi-Agent Systems (MAS), defined as systems composed of (or based on) a number of autonomous organizations that relate to each other – and with an environment – corroborating to solve a problem whose solution is beyond the individual capacity of each component (HÜBNER et al., 2004; WEISS, 1999; WOOLDRIDGE, 2001).

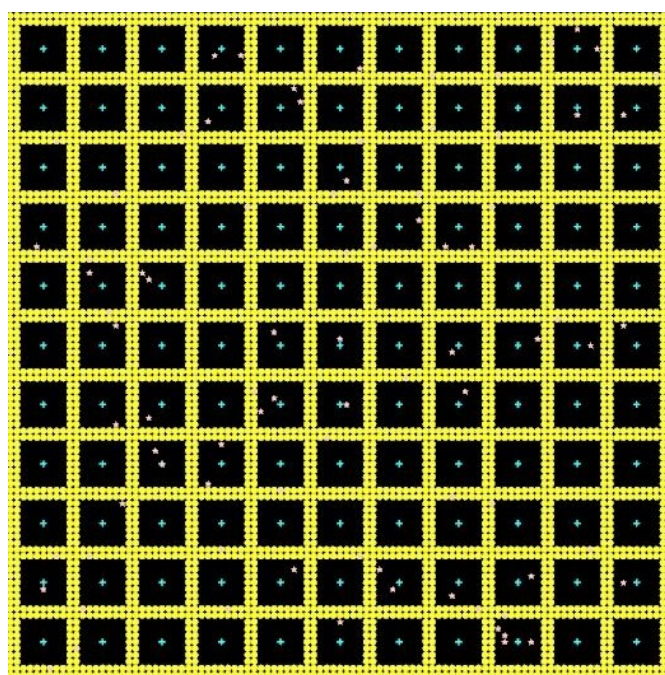
MAS aim the study of “community” highlighting the existing forms of interaction between the entities constituting the system (called agents) and its organization. This model has been used for research in computational environment of emergent behaviors of complex systems – such as the immune system (IS) – since they require to be modelled only: (1) the basic units that make up the system and (2) their relations, on the assumption that the collective behavior – complex – will emerge from the interaction of individuals (LI et al., 2009). Another important feature of the MAS is their ability to investigate hypotheses as cells – and mediators – relating to each other, in the manner that arise emergent behaviors from these interactions. Although MAS is the best choice for modeling the complexity of some systems, it requires high computational power when they present large amounts of agents, which can be a limiting factor

in its application (LI et al., 2009).

Some MAS focused on the investigation of the IS have been proposed, including (i) the BIS – The Basic Immune Simulator (FOLCIK et al., 2007) – designed to study the interactions between innate immunity cells and the cells of adaptive immunity and (ii) the *AutoSimune* – inspired by the BIS and designed by Possi (2012) and colleagues – originally designed for the investigation of autoimmune events (SILVA et al., 2012).

The *AutoSimune* was developed using the framework Repast Symphony – free, open source and widely used in agent-based modeling (NORTH et al., 2005) – with the *bottom-up* approach – which emphasizes the microscopic level, highlighting that the agents in this model are able to perceive the environment, act on it and store states. Each agent represents an entity of the real system and can be heterogeneous – each one with their own states and rules – being able to interact with others agents and environment (POSSI, 2012). The environment is divided into zones, which have been implemented based on the concept of projections offered by the framework. The selected projection for *AutoSimune* was the grid space, e.g., a discrete space with a predetermined number of rows and columns forming space cell, such as a matrix (Figure 2).

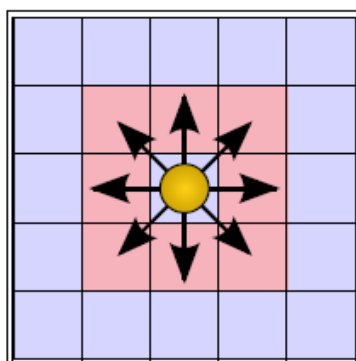
Figure 2 – Grid space in *AutoSimune*



Source: *AutoSimune* (POSSI, 2012)

The agents are found in any cell of the matrix and they have eight displacement possibilities: left, right, up, down and diagonal, which are represented by the pink cells (Figure 3). The movement is also allowed for all grid edges, which is a toroidal space. So if the agent moves to the right edge of the grid it will appear on the same line, however, at the left edge (POSSI, 2012).

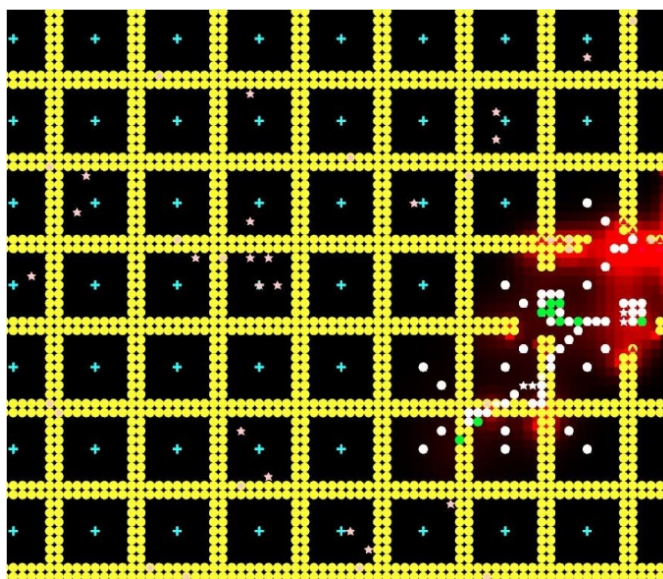
Figure 3 – Agent movement possibilities (yellow circle) during the time required for transition between environments (tick)



Source: *AutoSimmune* (POSSI, 2012)

In this model, the zones correspond to regions of the body where the cells carry out their roles and functions, each of which has data layers to store information of the substances produced / liberated space of each grid cell, such as cytokines. Currently there are some areas implemented in *AutoSimmune*, and the Tissue zone is the main one. Thus, it is possible to realize the parenchymal tissue cells (yellow circles), migration portals (blue crosses) – blood and lymph vessels –, macrophages (white circles), lymphocyte T cytotoxic (green circles), B cells (pink circles), dendritic cells (pink star), neutrophils (white star), pathogen – e.g., *Plasmodium* – (red triangle) and tissue damage (red area) (Figure 4). This area simulates a microscopic slice of the parenchyma of a generic body, the place where occurs the first contact between the body's cells and the causative agent, and also the infection (POSSI, 2012; SIQUEIRA-BATISTA et al., 2014).

Figure 4 – Area of image tissue, implemented in *AutoSimmune*

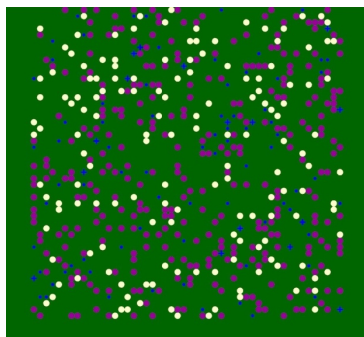


Source: *AutoSimmune* (POSSI, 2012)

The Lymph Node area refers to the node, and it is there that the antigens are presented to lymphocytes, facilitating their proliferation after antigen recognition (SOUZA, 2014). It is

possible to identify the T cells (circles), B cells (pink circles), the etiologic agents (blue circles) and portals (blue crosses) (Figure 5). According to Folcik et al. (2007), this area can also represent the spleen.

Figure 5 – Image of the Lymph Node zone in *AutoSimmune*



Source: *AutoSimmune* (POSSI, 2012)

Circulation area represents the lymphatic and blood circulation – in which are represented healthy red blood cells (white dots), infected erythrocytes (yellow dots) and pathogens (blue spots) – while BoneMarrow zone simulates an abstract bone marrow, which was created due to the need to represent the mechanisms related to central tolerance of B cells and that is responsible for creating the agents that simulated the same agents, with random specificities and which were tested by means of the mechanisms of central tolerance. The thymus was also simulated (SOUZA, 2014).

In *AutoSimmune*, the time is marked in ticks, which represent the interval required for the transition from one environment to the next state. Thus, all of the scheduled events should be executed and completed so that the next tick can occur, and so creating a synchronized simulation (POSSI, 2012; SIQUEIRA-BATISTA et al., 2014). In practice, during this period, all the agents change their positions, release substances and analyze their neighborhoods (all via a retrograde analysis of the previous tick). Once all agents run its full activity the tick is finished and their information are updated. Such behavior allows, from the “agent view”, that everything happens concurrently (POSSI, 2012). However, the real length of each tick differs and depends heavily on the machine’s processing power when it is simulating the model, but this point is not so relevant, unless when it comes to optimization. In this case, the tick in the model is (or simulate) an hour, a day, or a month in the real world. It is of utmost importance (POSSI, 2012).

AutoSimmune has been used to (1) investigate the role of mast cell in the control of inflammation (SILVA et al., 2012), (2) the immune response in the post-infectious glomerulonephritis by the bacteria *Streptococcus pyogenes* (BASTOS, 2013), (3) sepsis (SIQUEIRA-BATISTA et al., 2012b), (4) Chagas disease (FARAGO, 2014), besides the theoretical proposition of application for the study of *P. falciparum* malaria.

After this, should be observed that this work is not a research involving human subjects – in line with established pursuant to Resolution No. 466, of December 12, 2012 – and therefore was not subject to analysis and approval by the Committee ethics in Human Research (CEP).

It was proceeded the computational modeling of *Plasmodium falciparum* and the erythrocyte agent in *AutoSimmune* as described below.

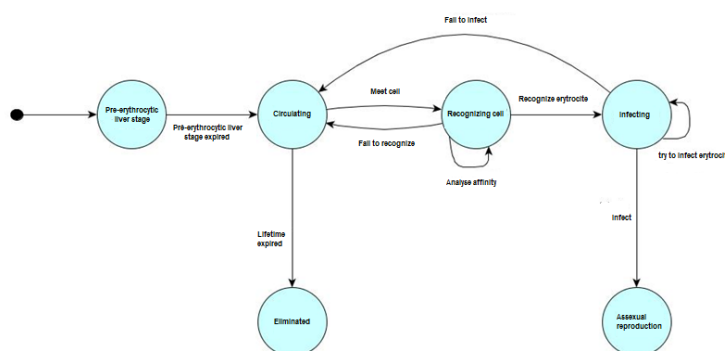
3 COMPUTER MODELING OF *PLASMODIUM FALCIPARUM* AND ERYTHROCYTE IN *AUTOSIMMUNE*

The starting point for computational modeling of *P. falciparum* and erythrocyte was the “structure” existing in *AutoSimmune* simulator. The choice of the etiologic agent was given for (1) the issues, already described, of relevance in clinical terms, and public health described in literature and (2) the previous experience of the authors with simulation of infectious processes that occur with marked systemic inflammatory response syndrome (SIRS) – for example, bacterial sepsis investigated by Sousa (2014) and Gomes et al. (2015).

Currently, such proposal is focused on the mapping of the main aspects of the immune response in sepsis for testing *in silico* hypotheses about this morbid condition in the case of Gram-negative bacteria (OLIVEIRA et al., 2012; SIQUEIRA-BATISTA et al., 2012c). From it, pathophysiological and therapeutic hypotheses have been raised, particularly with regard to the use of antimicrobials, and references to the characteristics of this morbid entity (SOUZA, 2014).

In this study, we used the framework Repast Symphony, in its version 1.2.0 for the Linux platform. From the established choices were characterised space and time of the interactions, as well as representations of cells, pathogens, cytokines and tissue. The implementation of *P. falciparum* and erythrocyte agents and the *AutoSimmune* was proceeded according to the state diagrams shown below (Figures 6 and 7).

Figure 6 – Rules of *Plasmodium falciparum* agent in *AutoSimmune*



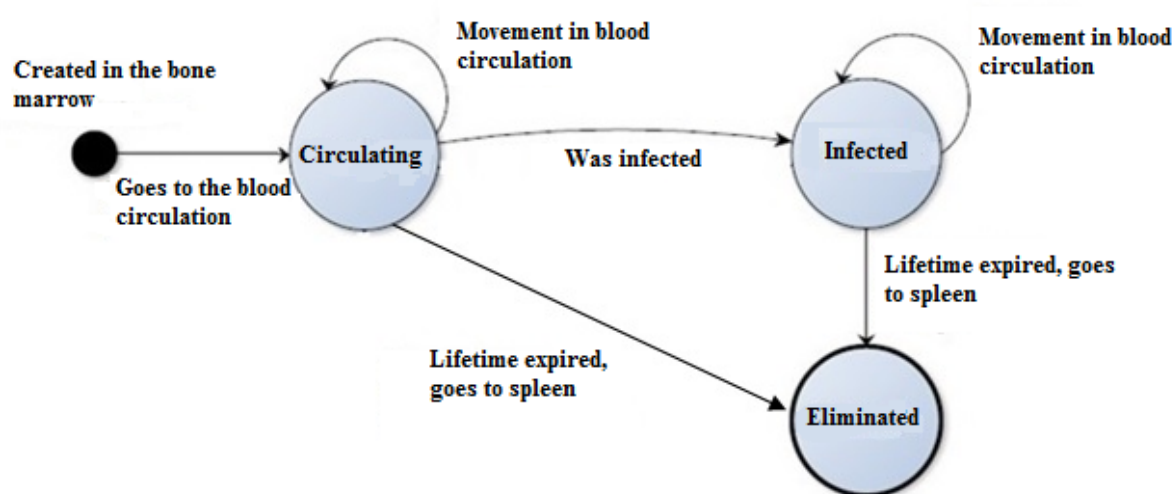
Source: *AutoSimmune*. Authors data

The practical functionality of the simulation is realized by a set of actions initiated from the time the agent appears in the fabric zone. When this occurs, the agent remains during a specified number of ticks in the stage of pre-state erythrocytes. When this period ends, the *Plasmodium* agent starts circulating in the environment. When it finds a cell, the *Plasmodium* agent tries to recognize whether it is a red blood cell (Figure 6). If the agent recognizes it as red blood cell, it enters in the “infecting” state in which it remains during a certain number of ticks

trying to infect the red blood cells (in the case of the experiments described in this article, the maximum time was 05 ticks). If the agent has been successful in generating infection, it enters in a state named “Asexual reproduction” in which, once again, it generates new agents. If the agent fails in the process of recognition and infection of the cell, it returns to the state “around”.

All the agent *Plasmodium* must contain the number of ticks which should be passed from the moment when it was created until the moment of execution. *P. falciparum* species should also contain a number of ticks that represents the half-life of parasites in the environment (05 ticks, as reported). If during the state “around”, the counted number of ticks per agent is greater than or equal to the number of half-life of ticks, the same is eliminated.

Figure 7 – Rules of agent erythrocyte in *AutoSimmune*



Source: *AutoSimmune*. Elaborated by authors

The implementation of the erythrocyte was made from its creation in the bone marrow (Figure 7). Thus, after this step, the cell reaches the bloodstream, where it remains circulating randomly. During this period, the cell is infected with *Plasmodium* agent or reaches its timeout (in the case of the experiments described in this article, were 50 ticks to erythrocytes). In both situations, the final destination of the erythrocyte is the elimination (as in the biological domain). In the event of infection, it keeps moving randomly in circulation until, time out, and it is directed to the spleen for disposal. However, when red cells are not infected by *Plasmodium* and the time limit is exceeded (as mentioned, 50 ticks), this cell is forwarded directly to the spleen and so it is also eliminated.

4 RESULTS AND DISCUSSION

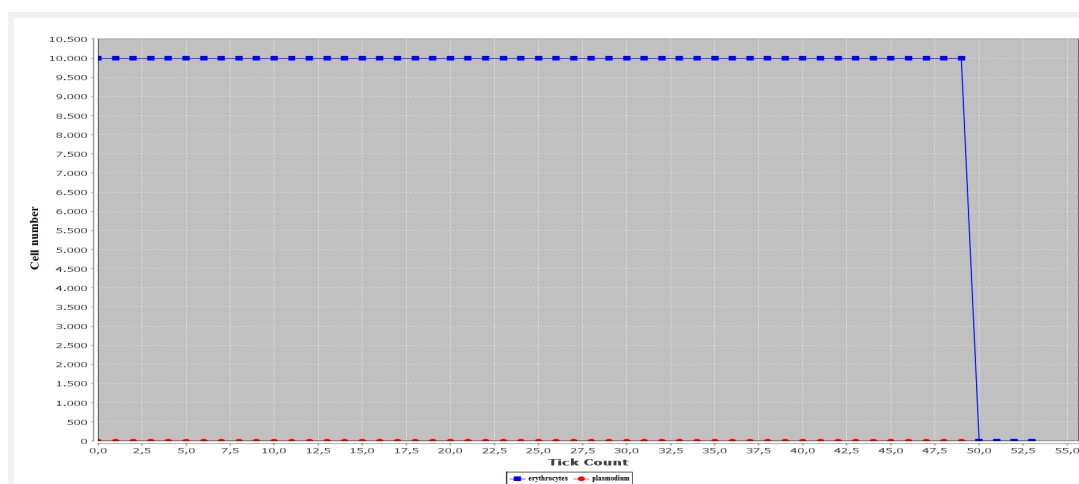
After the completion of the first version of the artificial immune system with the attributes of simulation of *P. falciparum* malaria, the experiments were designed *in silico* involving the response to different challenges as the variation of inoculum protozoa and red blood

cells, based on the literature (GOMES et al., 2012)(REY, 2008). The results in computational experiments are shown below, and related to current scientific literature, for assessing the ability of the simulator to produce data consistent with biological realities.

4.1 Experiment 1

First, it was decided by a simple test in an attempt to highlight the homeostasis model. Only red blood cells (10,000 cells) were included in the simulation and it was noted that, in the absence of pathogen (not able to release any inflammatory stimulus), the number of red blood cells remains constant over time. In the absence of the causative agent of malaria, there is no impact on the immune system, or in red blood cells (operational site of the protist), what made these cells remain from the figures in the simulator (Figure 8), up to 50 ticks, half-life of erythrocyte. We should comment that the test is far from the actual number of red blood cells of a human body, which varies according to age and sex, 4.6 to 6.2 million per ml of blood for men adults and 4.2 to 5.4 milion for adult women in venous blood. It is a consequence of the difficulty related to the computing power needed to run a system with a number of agents close to reality.

Figure 8 – Simulation inserting 10,000 erythrocytes with no associated etiologic agent



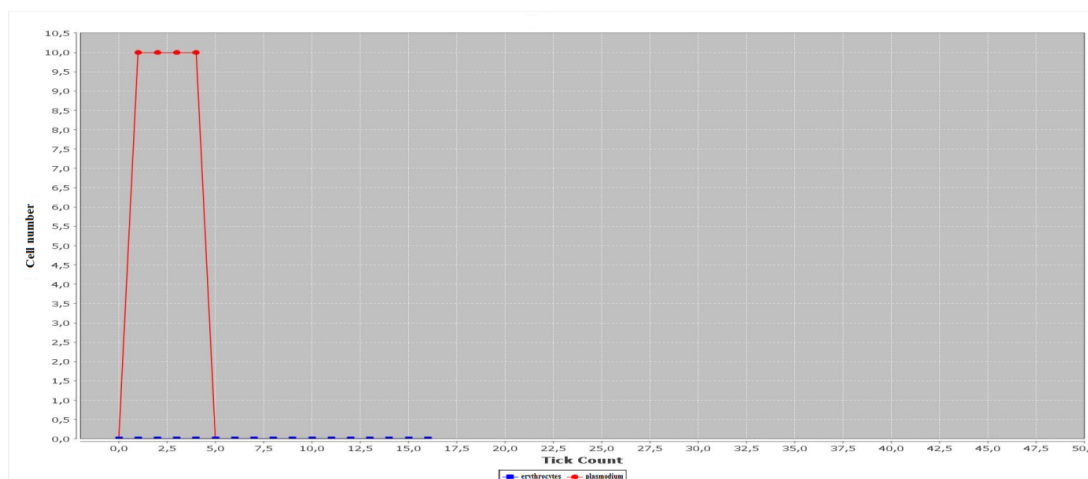
Source: AutoSimune

4.2 Experiment 2

In this test, we intend to go the other way relating to Experiment 1. We included only *Plasmodium* agents in circulation in a quantitative of ten agents and starting from the assumption that when there is no red blood cells to be infected the *Plasmodium* agent will not be able to reproduce and will die at the end of its life (Figure 9), e.g., after 05 ticks. This test matches

in vivo the impossibility of intrahepatic merozoites start their erythrocytic cycle. In humans the total absence of red blood cells is not feasible, and it is not compatible with life, but a parallel situation may be thought as the lack of receptors on erythrocytes for certain species of *Plasmodium* (REY, 2008), as seen in relation to the necessary presence of Duff system receptors for the occurrence of malaria by *P. vivax*.

Figure 9 – Simulation inserting five *Plasmodium* without erythrocytes



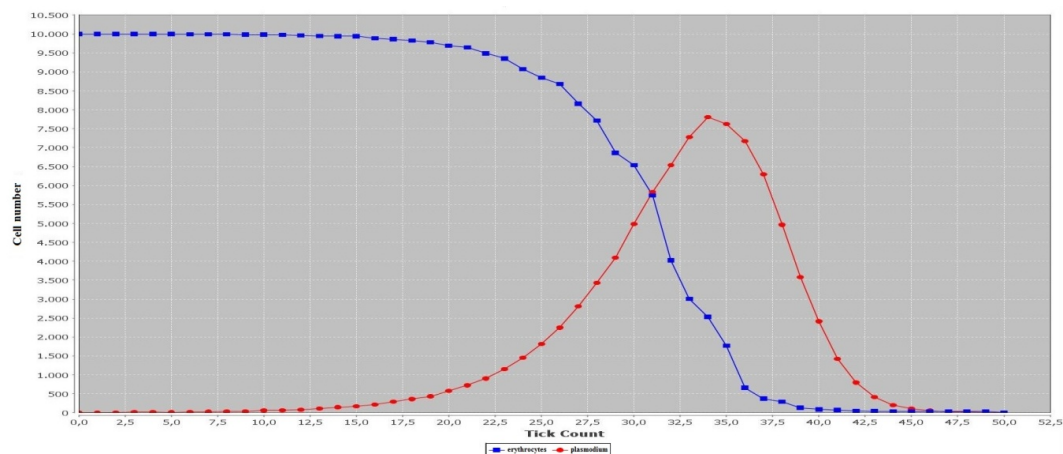
Source: *AutoSimune*

At this point, it is essential to comment that in natural infection with *Plasmodium*, experimental studies have shown (REY, 2008) that when there is inoculation of 20 sporozoites into the bloodstream in 30 to 45 minutes all of them have penetrated into the hepatic cells (in *AutoSimune*, Tissue zone), then they are named cryptozoites and it takes about five days for them – in *P. falciparum* malaria – to reach the form of liver merozoites. Each sporozoite gives rise to, at the end of hepatic schizogony, 40,000 liver merozoites, what ends the pre-erythrocytic cycle and begins the erythrocytic cycle. The pre-erythrocytic cycle happens in Tissue and is not visible in its phases, in *AutoSimune*. So we consider the preview occurrence of the cycle but it has not been established. What can be seen in the experiments is the erythrocytic cycle.

4.3 Experiment 3

A third test was conducted to demonstrate the behavior of 10,000 erythrocytes after an “inoculation” of five *Plasmodium* agents right at the beginning of the simulation. The results showed that the *Plasmodium* agents are capable of infecting erythrocytes maintaining a rapid multiplication and killing completely all the red cells (Figure 10). Such complete removal can be observed in the tick 50.

Figure 10 – Simulation inserting 10,000 erythrocytes and five *Plasmodium*

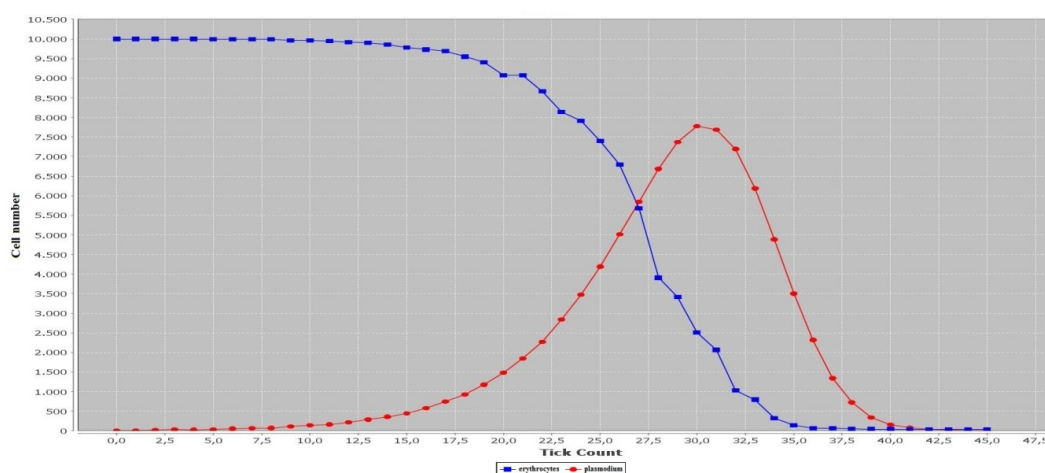


Source: *AutoSimmune*

4.4 Experiment 4

In this test – similar to the previous experiment (3) – the difference is the increase of the number of *Plasmodium* agents to 10 at the beginning of the simulation. The 10,000 red blood cells were retained. Thus, it was possible to see that, by increasing the number of *P. falciparum* the red blood cells are extinct faster than with 05 *Plasmodium* agents (by the tick 35). The peak of the agent was observed between the tick 30 and after some time, its quantity declined, since, in the absence of red blood cells multiplication of the *Plasmodium* agents lead to death (Figure 11).

Figure 11 – Simulation inserting 10,000 erythrocytes and 10 *Plasmodium*



Source: *AutoSimmune*

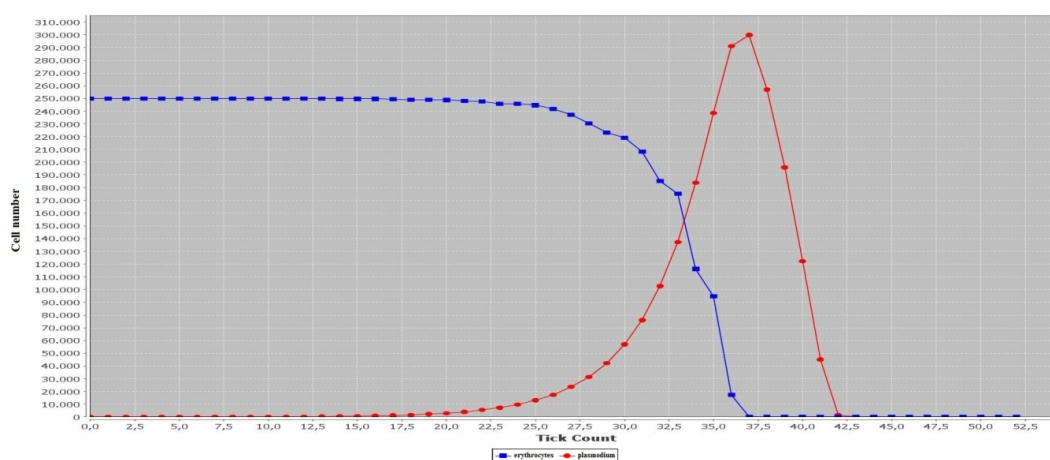
4.5 Experiment 5

After concluding that the *AutoSimmune* is able to provide experiments that show results consistent with the medical literature – infection of red blood cells by *P. falciparum* – we decided to make an attempt to approach these tests to the reality of the human infection.

The human blood presents about 5,000,000 red blood cells per mm³, with variations due to age and sex. The *Plasmodium falciparum* sporozoite (which is the most virulent species therefore chosen for the simulation) has its development cycle in the liver and comes to produce up to fifth or sixth days after inoculation of sporozoites, approximately 40,000 hepatic merozoites (REY, 2008). From this, they penetrate into the red blood cells and they will continue the biological cycle, establishing blood schizogony, and releasing new merozoites, each cycle within a range of 36 to 48 hours. At this time – when there is the destruction of red blood cells and release of the new agents in the circulation – there are pigments that act as exogenous pyrogen, triggering the immune response and consequent production and release of endogenous pyrogens, the interleukins (REY, 2008; SCHOLZEN; SAUERWEIN, 2016). Thus, there is fever, a characteristic sign of malaria (fever paroxysm).

As in humans, the vector *Anopheles* spp. usually inoculates an average of 20 sporozoites, at the end of the liver cycle; it can be obtained quantitative 800,000 merozoites, considering that each sporozoites that starts the liver schizogony cycle will rise to 40,000 merozoites. Considering that a human being has 5 million of red blood cells per milliliter of blood in a liter of blood would have to 5,000,000,000 (five billion) of red blood cells; so in five liters (average blood volume considered), we would have a total of 25 billion of red blood cells to be infected. Thus when establishing the equivalence in the simulator to the real situation, we would need to conduct an experiment with 250,000 red blood cells to be infected by eight parasites. The results of the simulation performed with these values are presented in Figure 12.

Figure 12 – Simulation inserting 250,000 erythrocytes and 8 *Plasmodium*



Source: *AutoSimmune*

In this graph it is possible to realize that it was spent a larger amount of ticks to erythrocytes and *Plasmodium* agents interact with each other. This situation is explained by the

significant increase in the number of programmed cells. It was noticed also, that as the number of *Plasmodium* was increasing, the erythrocyte number was declining, reflecting the stage where there happens the lysis of red blood cells and the onset of signs and symptoms in the biological reality. It is worth noting the high capacity of the *Plasmodium* agent replication, which in a small interval of ticks increased significantly their number. Moreover, the greater the number of parasites, the smaller the amount of red blood cells: in fact, (i) the maximum peak of *Plasmodium* (which reaches a population of 300,000) near the range (35 to 37.5) ticks and (ii) there is complete eradication of red blood cells – a situation that is incompatible with life – before the tick 37.5. After a few ticks in the absence of erythrocytes, the number of parasites also declines – since it is impossible to occur infections – with the complete elimination near the tick 42.5.

5 CONCLUSION

Despite decades of research on malaria caused by *Plasmodium falciparum*, several points of immunology remain unclear. With the implementation and modeling of *Plasmodium* agent and erythrocyte in *AutoSimmune*, it was studied the relation between the infectious agent and one of its target cells, besides highlighting similarities with those described in biological reality.

Thus, it is believed that the model approximates to the observed situation in real life. In addition, *in silico* research have demonstrated great potential to contribute to the knowledge of the mechanisms of illness of various diseases. The great expectations are about the discovery of a vaccine capable of reducing the occurrence of malaria, which would increase the quality of life of the populations affected by this disease. So it is remarkable the need for further research on the scope of computational biology and computational medicine.

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