A Multiagent-based Simulation of the Infection of the Macrophage by Trypanosoma Cruzi in the Acute Phase of Chagas' Disease: Influence of the Initial Inoculum and Protozoan Escape Factor

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Abstract: Chagas' disease presents a wide distribution in Latin America. Some epidemiological studies show that there are a prevalence of 16 to 18 million people affected by the disease and at least 100 million people at risk of being infected on these areas. Recently, due to the globalization, the disease, which was previously endemic only in countries of Central America and South America, is now presenting cases in other regions such as North America and Europe. *Trypanosoma cruzi*, the etiological agent, has the ability to promote changes in the tissues of the vertebrate host with significant morbidity, according to the degree of infection. This article presents a simulation of the human immune system using a multiagent system approach. More specifically, we propose a simulation study aimed at the acute phase of Chagas' disease, related to the macrophage-*Trypanosoma* interaction. The simulation showed that the initial number of *T. cruzi* influences in the outcome of infection and was found a relationship between the escape factor and the total elimination of *T. cruzi*.

1 INTRODUCTION

Chagas' disease (CD) was first described by Carlos Ribeiro Justiniano Chagas in 1909, in northern Minas Gerais (Brazil). Recently, because of the globalization, this disease that was endemic only in countries of Central America and South America is now occurring in other regions of the world such as North America and Europe (Siqueira-Batista et al., 2007; Rassi Jr et al., 2012). *Trypanosoma cruzi* (*T. cruzi*), the etiological agent, is capable of causing a significant damage in the vertebrate host tissues leading to serious consequences, according to the degree of infection (Alcantara and Brener, 1980). After infection, the individual develops an acute phase of short duration. At this stage a highly aggressive form of *T. cruzi*, the trypomastigotes, is present in the bloodstream. This causes the immune system response via humoral and cellular mechanisms of innate immunity such as complement-mediated lysis, natural killer cell cytotoxicity and phagocytosis carried out by the mononuclear phagocyte system (MPS), mainly by macrophages (Coura and Borges-Pereira, 2010; Rodrigues et al., 2010). The parasites use escape mechanisms to survive to the action of the macrophages and are not completely eliminated leading to a chronic disease characterized by low parasitemias with the prevalence of intracellular parasitism of the amastigotes form (Coura and Borges-Pereira, 2010; Rodrigues et al., 2010). The disease presents two distinct phases, an acute phase which is followed by a chronic phase that can be classified as indeterminate, cardiac or digestive form (Siqueira-Batista et al., 2007). Usually, one third of the patients presenting the indeterminate classification develop symptomatic CD some decades after the initial infection (Siqueira-Batista et al., 2007; Coura and Borges-Pereira, 2010).

It is believed that the *T. cruzi* load in the initial infection is a major factor in the development of CD (Borges-Pereira et al., 1988; Martins et al., 2012). Cases reporting feces of triatomine with more than 1500 parasites have been described, but it is believed that the quantities of *T. cruzi* needed to cause infection is far less than the inocula used in experimental infections (Coura and Borges-Pereira, 2010; Ro-

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drigues et al., 2010). Studies report that the vast majority of cases of acute CD in Brazil are asymptomatic or mildly symptomatic, possibly due to low inoculum and/or humoral response (Coura et al., 1983; Coura, 2013). A study encompassing almost three decades of patients follow-up showed that the cases of more severe chronic forms originated from cases with severe acute phase. Thus it is possible to deduce that the initial inoculum and/or the infecting strain of *T. cruzi* influenced the evolution of the disease. Experimental studies have shown that this load is generally low (Dias, 1982).

Among the cells of the mononuclear phagocytic system, the macrophage presents a key role in the host response to *T. cruzi*. These cells have trypanocidal and tripanostatic capacity and often determine the degree of susceptibility of the infected host (Russo and Starobinas, 1991). Activation of macrophages in the acute phase of CD has long been described in the literature. Activated macrophages are able to kill the parasite. However, the resident macrophages do not perform this action. The relevance of this trypanocidal activity is not yet fully known (Celentano and González Cappa, 1993). Macrophages phagocyte epimastigotes and trypomastigotes. The first kind is digested, while the latter kind escapes from the vacuole and is transformed into amastigotes that multiply inside the macrophages. This phenomenon occurs in the region where the penetration of trypomastigotes takes place, initiating the intracellular stage of the parasite. Each amastigote by binary fission yields dozens of new amastigotes that after four to six days are transformed into tripomastigotes that disrupt the cell and reach other tissues. A simple binary division of amastigote is repeated every twelve hours and produces about nine generations of parasites that disrupt the macrophage (Rey, 2008). Activation of macrophages leads to activation of T lymphocytes that secrete interferon γ, which in turn induces macrophages to produce interleukin 1 (IL 1), tumor necrosis factor (TNF), and nitric oxide (NO) (Siqueira-Batista and Geller, 2008). The NO is extremely toxic for the *T. cruzi* and seems to be responsible for tripanolitic action exerted by macrophages (Rey, 2008). More detailed studies to improve the understanding of the immune system may contribute to the treatment and prevention of the disease.

Multiagent system (MAS) has been successfully applied in many complex systems. It 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. Its main disadvantage, however, is the high computational cost when a large number of agents is used (hua Li et al., 2009). The simulation using techniques of multiagent systems (MAS) has been an important tool in the investigation of the phenomena of interactions between the various cell types of the immune system and pathogens such as viruses, bacteria, and protozoa (Possi et al., 2011; Folcik et al., 2007; Borges, 2012). These studies led to designs with a bottom-up approach, i.e., the systems are conceived defining the individual aspects of the agents, such that the emergency of the collective behavior occurs naturally from the interactions of the agents with themselves and with the environment. As a result, it is possible to test hypotheses of interest in a realistic manner, i.e., without programming the desired scenarios directly, but, instead, obtaining them as a consequence (Possi et al., 2011).

The group of Modeling and Simulation of the Immune System (ModeSimmune) of the XXX University, in a joint effort with the Departments of Medicine and Computer Science, is developing a simulator of the human immune system called AutoSimmune, based on the simulator initially developed by (Folcik et al., 2007). AutoSimmune has been developed since 2010 and is now able to perform many simulations of interest, including viral and bacterial infections and events involved in autoimmune conditions (Possi et al., 2011; Bastos et al., 2013). In this work, the investigations are focused on CD. To simulate CD regarding the interaction between *T. cruzi* and macrophage cells, it was necessary to model and introduce the *T. cruzi* agent. Furthermore, we had to modify the existing pattern of the macrophage agent in the simulator so that it was able to respond to stimuli from *T. cruzi* as described by (Coura and Borges-Pereira, 2010). We analyzed whether the initial number of *T. cruzi* parasites influences the outcome of infection and also the relationship between the rate of escape of the parasite from the macrophage phagocytic vacuole and the final number of *T. cruzi* agent after an intracellular multiplication cycle of them (Possi et al., 2011). Assuming that we have a reliable simulation of the real immune system, the analysis of the results that we have obtained from our *in silico* simulation provide us with strong evidences that new hypotheses on the investigation of potential therapies and/or drugs against CD might be of great impact in this field of study.

The next section describes some work related to the proposed system. Section 3 presents the material and methods used in the simulation, including a description of the model. Section 4 shows the results of running the simulation. Section 5 discusses the results presented. Finally, section 6 presents the conclusions of this work.

2 RELATED WORK

With regard to novelty, there have been other agent-based models used to study *T. cruzi*, including ((Galvão et al., 2008),(Devillers et al., 2008),(Galvão et al., 2010) and (Rogers et al., 2010)), but none have focused on the interactions of macrophages and *T. cruzi*.

(Galvão et al., 2008) developed of a two-dimensional agent-based model for chronic chagasic cardiomyopathy after stem cell transplantation. Their model included six different types of agents: inflammatory cell, fibrosis area, cardiomyocyte, proinflammatory cytokine tumor necrosis factor-α, *T. cruzi* and bone marrow stem cell. Although not explicit in the paper, they have used a model based on cellular automata.

(Galvão et al., 2010) developed a three-dimensional multi-agent-based computational model for the evolution of Chagas' disease. Their model included five different types of agents: inflammatory cell, fibrosis, cardiomyocyte, fibroblast, and *T. cruzi*. Note that the macrophages were not modelled. Fibrosis was a fixed agent and the other types of agents could move through the empty space randomly by using the Moore neighborhood. The aim of the model was to reproduce the acute and chronic phases of Chagas' disease and the volume occupied by all different types of cells in the cardiac tissue.

(Rogers et al., 2010) created a model-based agents focusing on the *T. cruzi* vector in the Texas region. They incorporated data related to the dominant sylvatic vector and host species for the *T. cruzi* in Texas into an agent-based model using NetLogo.

3 MATERIALS AND METHODS

AutoSimmune is an immune system simulator with original focus in autoimmunity. In its basic version, it simulates the bone marrow, thymus, lymph nodes, blood circulation and parenchymal tissue region. The regions are simulated as a discrete space in a form of 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 movement of the agent is done by changing its current position to a position in the *Moore neighborhood* (Dewri and Chakraborti, 2005). Thus, an agent cannot "jump" positions, it needs to move one position at a time. In a cell structure in the form of a two-dimensional grid, the Moore neighborhood (of radius one) comprises the eight neighboring cells to a central cell. If allowed in their specification, an agent can move from one region to another by means of special elements called portals, as proposed in (Folcik et al., 2007). The simulation of substances such as cytokines are performed by means of a layer of data, named ValueLayer, provided by the Repast framework[1], over which the simulator was built. Substances, as they are released by cells, undergo a process of diffusion, spreading in the surroundings of the site in which they were released, decreasing its concentration, and also undergoing a process of decay, reducing its amount with time (Possi et al., 2011). The ValueLayer is an abstract layer of data that, at the time of its creation, is associated with a region of the grid. It is possible to combine multiple layers of data at the same grid. Thus, an agent can know the concentration of a given substance at that time instant at position (i, j) (Possi et al., 2011).

The time is modeled using the concept of discrete unit time provided by the framework, called tick. Each component schedules their execution time, informing when to start and the invocation interval (Possi et al., 2011). As a result, all events are properly synchronized. 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 matching, the greater the affinity. For the calculation of 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 that are contained in A and B simultaneously, in the same order.

3.1 The Macrophage Agent Model

The software agent that plays the role of macrophages in the simulator AutoSimmune needed to be improved, since some of its functions that are essential in the interaction with the *T. cruzi* were not modeled and implemented in previous versions. The rules of this agent are illustrated by the state chart showed in Fig. 1.

The macrophages in AutoSimmune accomplish their activities in the tissue, represented in the simulator by the Tissue zone (based on "Zone 1" defined by (Folcik et al., 2007)) which represents a slice of a microscopic generic parenchymal tissue. This agent migrates to the tissue when they detect the presence of pro-inflammatory cytokines (MK1 group, after (Folcik et al., 2007)) and enter the Tissue zone. The

[1] repast.sourceforge.net

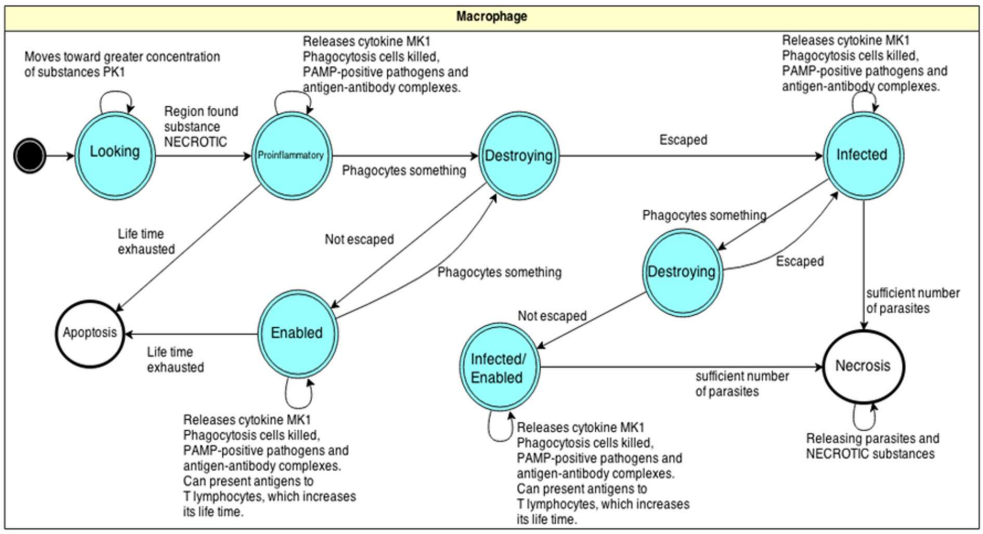

Figure 1: Macrophage state chart.

agent follows the cellular stress signaling substance until encountering the site of infection flagged by the necrosis substance released after cellular lysis. In the presence of necrosis, itcommutes to the macrophage pro-inflammatory state, which produces and releases cytokine MK1 and phagocytes dead cells, PAMP-positive pathogens and antigen-antibody complexes, i.e., antibodies bound to any antigen marking it for phagocytosis.

Changes in the macrophage behavior when it phagocytes some foreign particle were inserted in the AutoSimmune model. In this case, a transition state called Destroying was created. When a macrophage phagocytes *T. cruzi*, it may occur that *T. cruzi* is not destroyed and ends up infecting and multiplying inside the macrophage. If the micro-organisms escape from the parasitophorous vacuole and infect the macrophage, the macrophage switches to infected state, continuing with its actions until the number of parasites is large enough to rupture the macrophage.

After rupture, the cell dies and goes to the Necrosis state. At this time, the parasites are released into the medium and the dispersion of necrosis substance occurs until the macrophage agent is engulfed by another cell. When the micro-organisms or particles are destroyed the agent macrophage processes it, extracting their antigens and presenting them to T lymphocytes featuring the Enabled state. Infected macrophages that destroy any micro-organism will change to the Infected/Enabled state with the same functions of the Enabled state, but keeping the internal proliferation of pathogens.

3.2 The *Trypanosoma Cruzi* Model

Initially, *T. cruzi* infects and multiplies in cells of the mononuclear phagocyte system (MPS), notably macrophage. Later on, T. cruzi migrates to other parts of the organism and invades other host cells, especially smooth muscle, skeletal, cardiac nerve, enabling their stay in the host (Siqueira-Batista et al., 2007; Martins et al., 2012). As we want to simulate the primary immune response, we inserted *T. cruzi* in the Tissue zone. The representative agent of *T. cruzi* in AutoSimmune was modeled and implemented according to the parasite's main functions and features described in the biological literature, keeping the highest possible fidelity, so that we could emulate its behavior and allow the possibility for posterior *in vivo* analysis of the immune response towards the pathogenic agent and its peculiarities. The rules of this agent are illustrated in Fig. 2.

Initially, the agent is in trypomastigote form and randomly moves looking for other agents, which is called Circling state. When it meets another agent, *T. cruzi* checks if there is some affinity with it. If affinity exists, this is a possible host cell and so the parasite invades it. After invading the cell, *T. cruzi* turns into amastigote and initiates a cycle of multiplications by successive binary divisions with nine fissions mostly producing 50-500 parasites depending on the strain of *T. cruzi* and the host cell (Rey, 2008; Siqueira-Batista et al., 2007). After this step, still inside the parasitized cell, *T. cruzi* undergoes further transformation into the trypomastigote form and is released from the cell in order to penetrate into other cells and tissues.

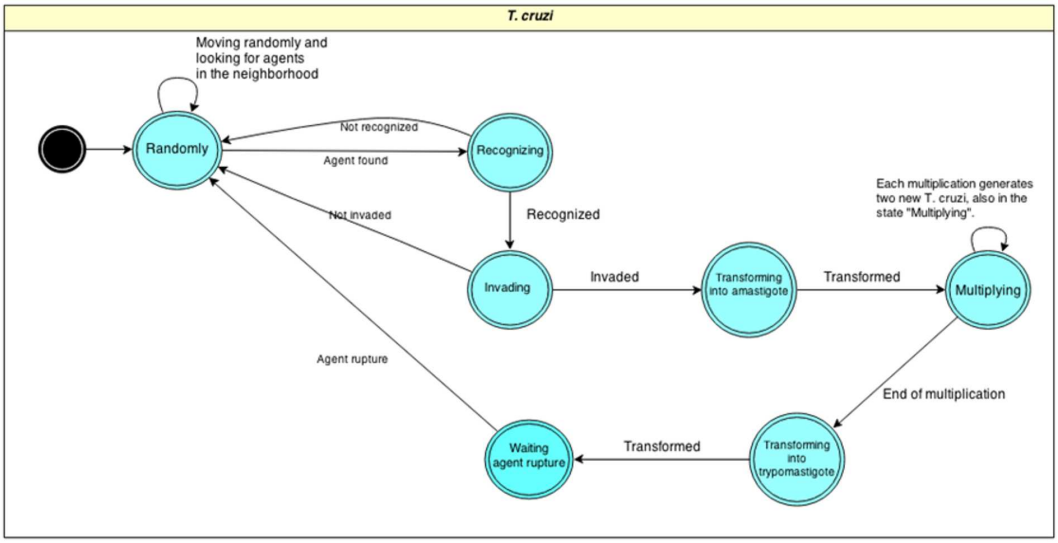

Figure 2: *Trypanosoma cruzi* state chart.

3.3 Definition of Variables

The environment for the interactions between agents in the simulations was the Tissue zone with grid size 150 by 150 units of space. To further analyze the interaction of the parasite with the immune system, a period of 1825 ticks was fixed. The proposed value for the tick in this study corresponds to six months of infection. In this simulated environment, according to the modeling decisions, the immune reaction should not happen effectively, since the secondary immune response has not been triggered yet and it is central to restrain the levels of parasites (Siqueira-Batista et al., 2007). During the simulations the following parameters were evaluated:

- Tick: Unit of time provided by the framework. The relationship between computational tick and real time required for analysis and interpretation of the simulations was based on the multiplication of *T. cruzi*. Amastigotes multiply by binary fission every 12 hours *in vivo*, according to Rey (2008), and in AutoSimmune the same process occurred within 5 ticks, so we defined that a tick corresponds to 2 hours and 24 minutes.
- Initial number of *T. cruzi*:Total number of *T. cruzi* inserted in the Tissue zone two ticks after the beginning of the simulation. This inoculated parasite load was set to 300 in the first series of simulations and 3000 in the second series.
- Initial number of macrophages: Number of macrophages present in the Tissue zone before the parasite load being inserted.
- Escape factor: Defines the chance of *T. cruzi* escaping from the phagocytic system and multiplying within the macrophage. It can vary from zero to one hundred percent of chance.
- Active infection: It is the active entry with energy expenditure of *T. cruz*i into macrophages. As the main mechanism of invasion occurs by phagocytosis, the chance of occurring active infection has been fixed at 0.10 percent.
- Breach number: The maximum number of parasites within the macrophage after multiplication cycle. When the number of *T. cruzi* reaches this limit, the cell breaks down and the parasites are released. In this work, we follow what is described by (Rey, 2008), i.e., after invading macrophage, *T. cruzi* multiplies through a series of binary divisions, about nine, generating approximately 500 strains.
- Tick when the number of *T. cruzi* is zero: The time when the number of parasites reaches zero.
- Maximum number of *T. cruzi*: It is the maximum number of parasites in the Tissue zone area at a given time. Generally, *in vivo*, this moment occurs in the acute phase of the disease during the first weeks of infection.
- Maximum number of macrophages: The highest occurrence of macrophages in the Tissue zone. Although initially there is a fixed number of macrophages, when the parasites are introduced, infecting and destroying cells, more macrophages are recruited to the site of infection. The amount depends on how the infection spreads through the Tissue zone and not necessarily on existent amount of *T. cruzi*.

- Number of *T. cruzi* at the end:Amount of *T. cruzi* in Tissue zone at the end of 1825 ticks.
- Number of Macrophage at the end: Amount of macrophages in the Tissue zone at the end of 1825 ticks.

4 RESULTS

In order to validate the modeling and to study the interactions between T. cruzi infection and the immune system, two sequences of simulations varying the escape factor were performed, one with initial number of *T. cruzi* equal to 300 and another with this parameter equal to 3000. These values were based on (Borges, 2012; Coura and Borges-Pereira, 2010) review to investigate the variation of inoculums *in vivo*. The values chosen for the escape factor were based on its relationship with the number of *T. cruzi* at the end. Each experiment has an increment of 0.1 or 0.01 in the value of the escape factor of the previous experiment. The range of 0.01 was used when the results showed a random pattern. On the other hand, the value of 0.1 was used when the experiments behaved with a more predictable pattern. Values above 1% were not simulated because it has been observed that in such cases, it is difficult for the macrophages to restrain the infection.

The purpose was to investigate three important issues in the study of CD: Whether there is a situation of aggression and inflammation in response to the parasite, as described in the literature by (Siqueira-Batista et al., 2007; Rochitte et al., 2007); Whether the initial number of *T. cruzi* influences the outcome of infection, as demonstrated experimentally in (Borges, 2012); and what is the relationship between the escape factor and the total elimination of *T. cruzi*. Tables 1 and 2 show the results obtained after 104 simulations, 52 for initial number of *T. cruzi* equal to 300, and the remaining for initial number of *T. cruzi* equal to 3000, respectively.

With inoculum of 300 *T. cruzi* agents, the macrophages eliminated all of them in 20 simulations with maximum escape factor up to 0.52%. For the inoculum of 3000 in 52 simulations, this phenomenon occurred 13 times with maximum escape factor of 0.37%.

In simulations with inoculum of 3000 *T. cruzi* agents, the lowest escape factor where the number of *T. cruzi* at the end did not reach zero was 0.24% and the highest escape factor where the number of *T. cruzi* at the end zeroed was 0.37%. This is a narrow range (around 0.13%) where the outcome of the infection varies.

Table 1: Simulations with inoculum equal to 300.

escape factor(%)	Tick T. cruzi=0	max. T. cruzi	T. cruzi tick 1825	Mϕ tick 1825	max. Mϕ
0.00	248	1001	0	364	369
0.10	166	1020	0	438	440
0.20	208	1000	0	348	352
0.21	259	3779	0	396	403
0.22	208	830	0	340	342
0.23	123	813	0	353	354
0.24	345	1000	0	343	348
0.25	316	1115	0	374	376
0.26	195	804	0	336	337
0.27	-	6526	6	373	399
0.28	121	799	0	349	349
0.29	419	1269	0	330	339
0.30	-	23064	7	1395	1396
0.31	-	4330	14	854	854
0.32	279	1555	0	426	430
0.33	1205	1221	0	475	477
0.34	1766	2085	0	489	494
0.35	322	832	0	424	430
0.36	-	25272	3655	2230	2248
0.37	1757	1204	0	443	446
0.38	-	33981	8406	1526	1537
0.39	-	95795	30648	1086	1479
0.40	-	34447	3782	1674	1674
0.41	723	1556	0	419	426
0.42	-	124231	38481	3078	3097
0.43	-	88893	26064	1020	1471
0.44	144	798	0	308	310
0.45	-	95426	11837	1267	1267
0.46	425	868	0	287	300
0.47	-	20003	3433	1804	1804
0.48	-	85131	17386	1092	1515
0.49	-	11000	427	1221	1236
0.50	-	122758	60693	2659	3030
0.51	-	61358	15387	1928	2069
0.52	358	799	0	310	311
0.53	-	105997	22354	2842	3627
0.54	-	132487	30863	1870	2698
0.55	-	186631	101125	4574	5874
0.56	-	91251	39787	2590	2590
0.57	-	132072	47724	4837	5743
0.58	-	60626	18765	1128	1194
0.59	-	67964	24631	746	1531
0.60	-	6432	1425	823	831
0.61	-	2206	47	602	608
0.62	-	149570	90749	2478	3332
0.63	-	59905	27439	3927	3966
0.64	-	117307	15611	678	2239
0.65	-	9386	3829	1474	1474
0.70	-	174423	32523	3182	3589
0.80	-	107358	46055	2627	2900
0.90	-	147222	68838	3034	3806
1.00	-	148243	133889	4332	5149

There is a greater tendency showing that the largest the escape factor the largest the probability of *T. cruzi* to multiply leading to an increasing number of parasites in the environment, as observed in chart 3. This occurred in the simulations of this study though

Table 2: Simulations with inoculum equal to 3000.

escape factor(%)	Tick T. cruzi=0	max. T. cruzi	T. cruzi tick 1825	Mϕ tick 1825	max. Mϕ
0.00	179	5502	0	425	425
0.10	211	3869	0	343	343
0.20	471	4019	0	491	507
0.21	1168	4516	0	406	419
0.22	959	5830	0	450	463
0.23	1247	4030	0	319	341
0.24	-	4290	3	518	523
0.25	413	3640	0	386	391
0.26	955	3803	0	525	525
0.27	1174	4601	0	630	650
0.28	1477	4414	0	615	620
0.29	-	11574	10	726	746
0.30	-	18928	1	981	981
0.31	1815	5269	0	629	636
0.32	-	3574	1	498	499
0.33	-	5183	4	607	638
0.34	1624	4503	0	606	624
0.35	-	69472	5421	1345	1424
0.36	-	36352	283	2794	2845
0.37	648	3595	0	408	409
0.38	-	114117	6382	733	1736
0.39	-	123258	64354	2442	3649
0.40	-	9987	334	1917	1917
0.41	-	87750	7292	2533	2533
0.42	-	127525	10635	1755	1755
0.43	-	120302	10790	1148	1696
0.44	-	102800	43675	3173	3471
0.45	-	93822	20017	1319	1514
0.46	-	137813	14931	2579	2591
0.47	-	87098	15687	1420	1585
0.48	-	7586	840	1712	1735
0.49	-	50139	5537	3423	3423
0.50	-	86034	24686	1803	2327
0.51	-	145739	12674	1523	2187
0.52	-	184655	96294	5319	6856
0.53	-	25522	9219	1627	1651
0.54	-	181202	68261	6731	6731
0.55	-	58513	22986	2233	2511
0.56	-	62830	15419	890	1115
0.57	-	156409	60189	2914	4690
0.58	-	127694	25559	930	2776
0.59	-	188053	58005	1406	2919
0.60	-	117632	17953	1412	2113
0.61	-	87890	13474	787	1441
0.62	-	85169	85169	6838	6875
0.63	-	106039	45118	1980	2198
0.64	-	129197	79346	5794	5911
0.65	-	91751	28611	2663	2663
0.70	-	113388	51583	4487	4781
0.80	-	130328	72959	4799	5046
0.90	-	158986	88700	2710	4788
1.00	-	109838	63082	3380	3427

this event did not happen uniformly (charts 4 and 5).

In the simulations with inoculum of 3000, the lowest maximum number of *T. cruzi* was 3574 and it occurred for an escape factor of 0.32%, while the highest number of *T. cruzi* was 188053 with an escape factor of 0.59%.

5 DISCUSSION

We observed that, in the presented simulations, the results did not differ significantly from *in vitro* studies. In this work, the initial number of *T. cruzi* influenced the outcome of infection. Experimental studies with different species of insects showed that the number of parasites deposited at the site of infection and that actively penetrate the host at the time of suction and defecation by these vectors is variable. After the contact between infected triatomines and humans, the outcome will depend on variables that control the chances of infection (Coura and Borges-Pereira, 2010; Rassi Jr et al., 2012; Coura, 2013) Some studies report that variables such as the infection rate of triatomines, the time between the sting and defecation, the number of evacuations and the quantity during this time interval, the number of parasites eliminated, the percentage of infecting forms and their capacity of penetration, and the intensity of the itching during the sting are of great relevance to the occurrence of the infectious process (Coura and Borges-Pereira, 2010). (Borges-Pereira et al., 1988) observed that among eight species of triatomines infected with *T. cruzi*, the mean number of parasites per evacuation was 140. In their study, the authors found an average of 232 parasites per evacuation among the P. megistus species. In our simulation study, besides the use of 3000 agents, we also used 300 *T. cruzi* agents, which is similar to the study of (Borges-Pereira et al., 1988).

The results of this study support the assumption that the escape factor and the total elimination of *T. cruzi* are closely linked, since variations of tenths and even hundredths in escape factor had a significant effect on the outcome of the simulations of the *T. cruzi* macrophage interaction in the acute phase of CD.

As might be expected, the secondary immune response is very important to prevent infection by the parasite that causes CD since in most simulations only the macrophage trypanocidal action was not enough to halt the progression and action of the parasite. Still, even not sufficient, the macrophages were able, in cases where the escape factor was very low, to eliminate parasites, and in other cases detain the spread of infection by stabilizing the amount of parasites. It shows the macrophage importance in modulating the immune response to this disease.

As noted by (Possi et al., 2011), AutoSimmune is still in development. However, the simulator has been used in an others studies with promising results (Da Silva et al., 2012; Bastos et al., 2013), which in-

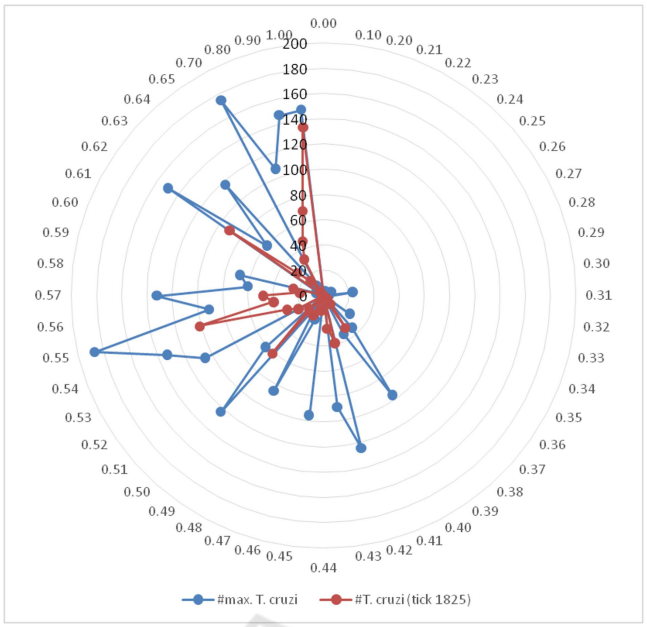

Figure 3: Relationship between escape factor and maximum number of *T.cruzi*/Number of *T. cruzi* at the end to inoculum equal to 300.

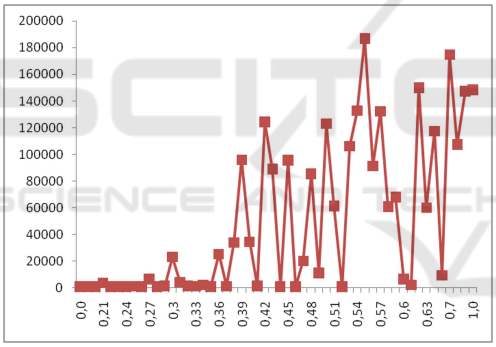

Figure 4: Relationship between escape factor and maximum number of *T. cruzi* to inoculum equal to 300.

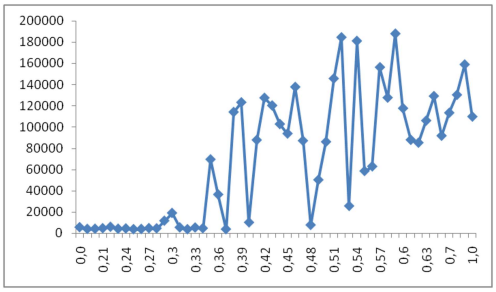

Figure 5: Relationship between escape factor and maximum number of *T. cruzi* to inoculum equal to 3000.

creases the expectation that it will be possible to refine our model, enabling the creation of a tool that supports hypothesis testing in biology and indicates new insights for *in vitro* and *in vivo* research.

6 CONCLUSION

We conducted a study of an *in silico* simulation of interactions between *T. cruzi* parasites and macrophage cells, in the AutoSimmune. We did not find similar studies in a wide search in PubMed about *in silico* simulation of this interaction. The study presented in this paper showed that the *in silico* model of the interaction between *Trypanosoma cruzi* and macrophage is a plausible approximation to what is described in the medical literature (Siqueira-Batista et al., 2007). We could observe that the initial number of *T. cruzi* parasites influences in the outcome of infection, as demonstrated experimentally, and found a relationship between the escape factor and the total elimination of *T. cruzi*.

Observing the results for the number of *T. cruzi* equal to 300 and 3000, we noticed the importance of the quantity of inoculated strains on the outcome of Chagas' disease. As in (Borges, 2012), it was observed that the tissue parasite load is directly related to the inoculum used for the infection.

In addition to the results that support the hypothesis about the quantity of inoculated strains on the outcome of Chagas' disease and relationship between the escape factor and the total elimination of *T. cruzi*, other important contributions of this research are a computational model for the acute stage of Chagas' disease and models of behavior for the macrophage and *T. cruzi*.

As future work, we intend to compare results of analysis *in silico*,*in vitro* and *in vivo* as well, to obtain a consensus measure of the value of the escape factor, and its relation to the activation of the secondary immune response, due to its importance (Siqueira-Batista et al., 2007).

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