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Artificial macrophages and the human immune system computational modeling for the investigation of sepsis pathophysiology: Perspectives*

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Resumo

A sepse representa um evento mórbido de extrema importância do ponto de vista clínico e de saúde pública, atingindo milhares de pessoas anualmente, no mundo. De uma perspectiva imunológica, os macrófagos são células extremamente importantes na interação *Homo sapiens sapiens/* bactérias, ainda que muitos aspectos da atuação dessas células permaneçam aguardando elucidação. O presente artigo apresenta os requerimentos para a simulação computacional do macrófago no sistema *AutoSimmune*, permitindo o desenvolvimento de estudos sobre o papel dessa célula na sepse.

Palavras-chave: Sistema imunológico. Macrófagos. Modelagem computacional. Informática. Informática médica.

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Abstract

Sepsis represents a morbid event of fundamental importance both from clinical and public health point of view, affecting thousands of people worldwide annually. From the immunological perspective, macrophages are extremely important cells in the interaction *Homo sapiens sapiens* / bacteria. However, several aspects concerning the action of these cells await elucidation. This paper presents the requirements for computational simulation of macrophage in the *AutoSimmune* system, allowing the development of studies on the role of this cell in sepsis.

Keywords: Immune system. Macrophage. Computational modeling. Computer science. Medical computing.

1 INTRODUCTION

Sepsis–systemic inflammatory response syndrome, related to a suspected or confirmed infection–still holds great magnitude and epidemiological relevance in the context of infectious diseases worldwide. The recent incorporation of computational simulation–*in silico* experiment–has helped to understand the intricate mechanisms of the interaction microorganism / man (SIQUEIRA-BATISTA et al., 2014), aiming to expand knowledge and build scientific hypotheses with the effectiveness and resolvability compatible with contemporary medical practice (SIQUEIRA-BATISTA et al., 2012a). In addition, *in silico* experiments are less costly and render bioethical questions related to research with human beings less insurmountable (BASTOS, 2013; POSSI, 2012).

Based on these assumptions, this paper presents the outlines for the simulation of the macrophage in a multi-agent computer system: *AutoSimmune*.

2 MACROPHAGE IN SEPSIS

Macrophages are constituents of the mononuclear phagocyte system. These cells are derived from monocytes that are, in turn, originated from stem cells in the bone marrow. Some elements participate in their process of biological development, particularly GM-CSF growth factor and interleukin 3 (IL-3). The presence of growth factors in the bone marrow leads the progenitor cell to undergo proliferation and consequent differentiation, giving rise to cells known as promonocytes. After some divisions, promonocytes give rise to monocytes. However, for the stem cells to differentiate, it is necessary that promonocytes express in their surface membrane receptors for specific cytokines. Monocytes remain in the bone marrow for less than 24 hours. After this interval, they reach the bloodstream and are directed to several body tissues and organs (CASTILLO et al., 2009). In this way, macrophages are originated from blood monocytes that migrated to the tissues, or are originated from their own proliferation that occurs from in situ precursors. These cells constitute a heterogeneous group due to diversified phenotypes and may be found in different tissues as lymphnodes, spleen, bone marrow, perivascular connective tissue, serosa, lungs, liver, bones, central nervous system, synovial and cutaneous connective tissue (GREER et al., 2009). The process of differentiation, from monocytes into macrophages produces several changes, such as (CASTILLO et al., 2009): (1) cell size increases 5 to 10 times; (2) organelles increase in number and complexity; (3) phagocytic activity begins; (4) high concentrations of lytic enzymes are produced; (5) soluble mediators, responsible for different functions, are released.

Macrophages are big cells – with diameters of approximately 15 to 80 μm – irregular in shape. Their motility is comparable to that of monocytes in the bloodstream. Filiform

pseudopods can be observed in the cell considerably often. Their cytoplasm is abundant, blue colored, containing vacuoles and blue granules. The nucleus has an ovate outline, either indented or elongated. The elongated case when stained by Wright's method shows chromatin with a spongy aspect, while the nuclear membrane appears distinctively (GREER et al., 2009).

Macrophages take part in innate immunity, since they are responsible for the natural initial response against microorganisms. This response is given through phagocytosis of foreign bodies, such as bacteria, several substances, or residual tissues, that succeed in penetrating the organism (CASTILLO et al., 2009). Macrophages are able to build an answer to chemical stimulation of the microenvironment and to acquire functions consistent with the requirements of the tissue in which these cells are. This process is known as activation, it occurs when there are polymicrobial infections and the constituents of bacteria, represented by the lipopolyssaccharide (LPS) of gram-negative bacteria, lipoprotein acid (LTA) of gram-positive bacteria, zymosan (ZYM) from fungi and bacteria DNA, all of which bind to several toll-like receptors (TLR), thus activating the macrophages (ELLABAN; BOLGOS; REMICK, 2004).

The interaction between macrophages and the external environment takes place by means of several membrane receptors. When these receptors are occupied by agonists, deep changes are induced in the macrophage, causing the cell to migrate toward a substance, in the process known as chemotaxis. Movements arise in the membrane, directed to the contact receptors, so that adhesion, migration and phagocytosis take place. Finally, the synthesis of enzymes that are useful for antimicrobial activities occurs, enabling the activation of the macrophage (BRASIL et al., 1999). Their key triggering mediators are pro-inflammatory cytokines – interleukins (1, 2, 6, 8, 12), interferons and necrosis factors – lipopolisaccharides (LPS) and colony-stimulating factors (CSFs) – responsible for the recruitment of neutrophils to the site of infection and for cleaning bacterial infection. Anti-inflammatory mediators – receptor agonist for IL-1 (IL-1Ra), IL-10 and TGF-β – are subsequently produced by macrophages with the purpose of adjusting the production of inflammatory mediators (ELLABAN; BOLGOS; REMICK, 2004). The functions performed by macrophage cover the following processes (GREER et al., 2009): (1) Promoting or inhibiting inflammation; (2) furthering tissue repair and regeneration; (3) acting as tumoricid and microbicid; (4) taking part in innate and adaptive responses. These are cells able to produce mediators such as prostaglandins, leukotrienes, platelet activation factors, interleukins 1, 6 and 12, obtaining as a result the attraction of several cells to the site of infection with the achievement of an immune response.

When a bacterial infection occurs in a previously sterile site, macrophages have the role of detecting the pathogen and triggering the inflammatory response. After the pathogen is phagocytized, the release of pro-inflammatory cytokines – IL-1β, IL-6, IL-8 and tumor necrosis factor α (TNF-α) – takes place. These cytokines will recruit the constituting cells of inflammatory response (*i.e.*, neutrophils) aiming to combat the aggressor locally. During the process of phagocytosis, the macrophage processes and presents antigens on its surface, facilitating the recognition by T helper lymphocytes. These cells, in turn, give rise to the synthesis of

lymphokines activators of B lymphocytes, stimulating the production and liberation of antibodies specific to the antigens presented by macrophage. These antibodies bind to the antigens of microorganisms or cells infected by virus, avidly attracting other macrophages to phagocytize them (CASTILLO et al., 2009).

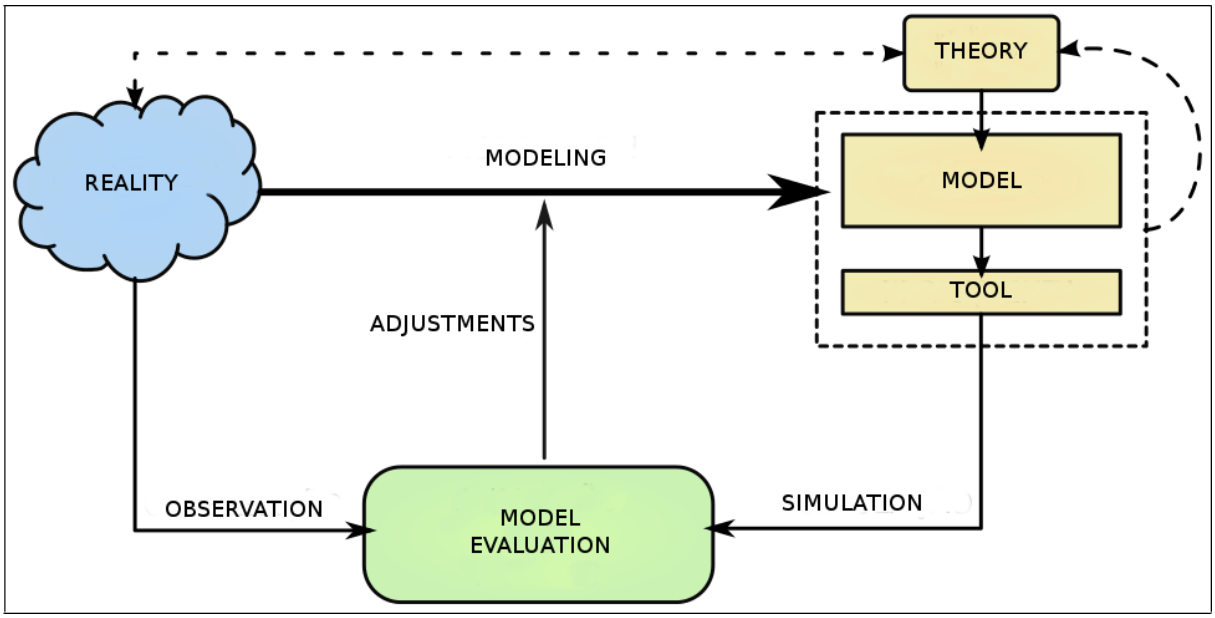
Such characteristic of macrophages is known as *classic activation* or M1. On the other hand, once started the resolution of this process, macrophages acquire anti- inflammatory action, with an increase in the expression of cytokines IL-10 and TNF-β, in addition to start a process of tissue repair – a characteristic called *alternative activation* or M2 (LIU et al., 2014). In M1 inflammatory macrophages, the effect of polarization is performed by the Th1 cell, or *natural killer*, derived from IFN-gamma, along with TFN-α. These cells are known to have the capacity to: (1) display activated pro-inflammatory action; (2) be involved in host protection and in antitumor immunity; (3) express several pathogen recognition receptors, and (4) produce pro-inflammatory cytokines, reactive oxygen species (ROS) and nitric oxide (NO). M2 phenotype macrophages, on the other hand, are stimulated by IL-4 and IL-3, originated in Th2 cells, or by macrophage colony-stimulating factor (M-CSF). These macrophages are involved in immune suppression and wound healing, justifying their description as wound healer macrophages (GORDON, 2003). Such functional duality of macrophage is called plasticity, highlighting its crucial role in the context and evolution of sepsis (BISWAS et al., 2012).

During the initial period of systemic inflammatory response syndrome (SIRS), the infectious process triggers an increase in circulating neutrophils and monocytes in hyper-reactivity (SIQUEIRA-BATISTA et al., 2014). As a result, there is an important increase in cytokines, what may lead to major consequences, like vascular damage and organ failure, which are severe outcomes that usually affects the septic patient. The main inflammatory mediators released are adhesion molecules (selectin, integrin and immunoglobulin); nitric oxide (NO); the metabolic products of arachidonic acid, like thromboxane A2, prostaglandins and leukotrienes; and the platelet activating factor (PAF) (BRASIL et al., 1999). However, due to their permanence in a hyperenriched environment, macrophages become irresponsive to toxins–a phenomenon called *tolerance to endotoxins*–responding with macrophage activation type M2. Such activation prevents endotoxin-tolerant cells to produce pro-inflammatory cytokines, leading the individual with sepsis to a state of immune suppression and death.

3 IMMUNOLOGICAL INVESTIGATION BY MEANS OF MULTI-AGENT SYSTEMS

The advancements in computer technologies allowed the emergence of *in-silico* experiments, which are faster, less costly and able to avoid the ethical questions involved in *in-vitro* and *in-vivo* experiments.

Figure 1 – Process of *in-silico* experiments



Source: Adapted from DROGOUL AND FERBER, 1992

In this research method, an initial model is built according to the existing knowledge and based on the observation of the phenomena to be studied. Next, by means of a simulation tool, several hypothesis are tested. The results of such simulations are then compared with experimental observations of the real phenomenon, either validating or indicating the need to adjust the model, additionally providing clues to improve the scientific theory about the studied phenomenon. This process is illustrated in Figure 1. Once validated, the model can be used to approach – with certain accuracy – the real phenomenon, thus being able to test hypothetical solutions for diverse problems.

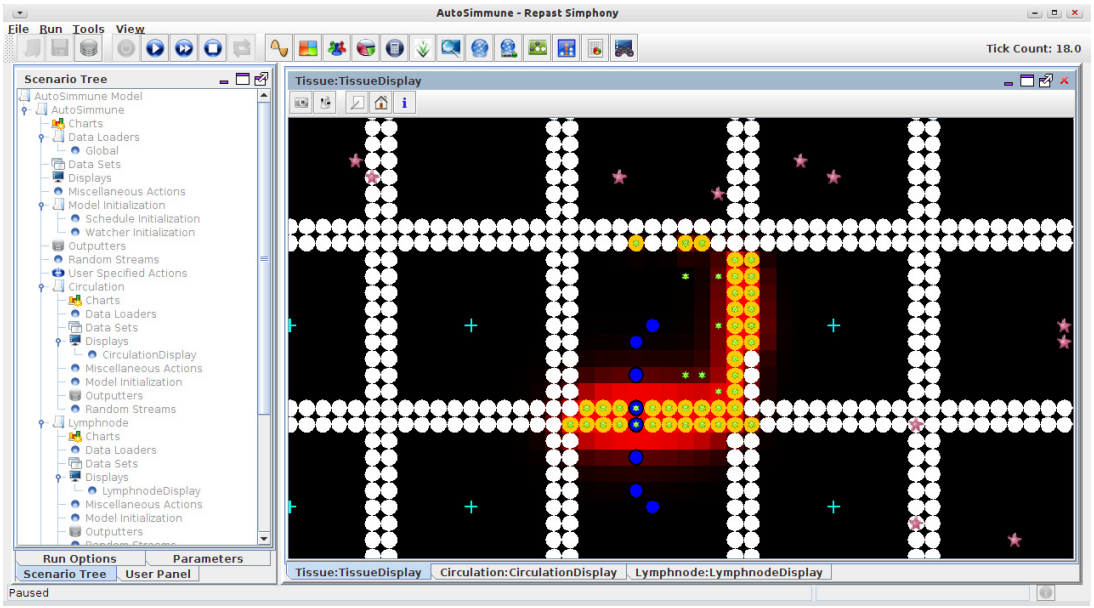
Multi-Agent Systems (MAS) are a possible methodology for building the model to be simulated and have been used in computer simulation of behaviors emerging from complex systems – such as the immune system (IS) – since they demand only the basic entities that make up the system and their relations to be modeled, assuming that the collective behavior will emerge from the interaction among elementary events. Some MAS geared towards the investigation of the IS have been proposed such as BIS – The Basic Immune Simulator (FOLCIK; AN; OROSZ, 2007) – developed to study the interactions between innate immunity and adaptive immunity cells, and *AutoSimmune*, proposed by Possi (2012), inspired by BIS but primarily focused on the investigation of autoimmunity.

AutoSimmune uses the framework Repast Simphony [7] as simulation tool. It is important to notice that the agents in this model are reactive to state, being able to perceive the environment – perceive other agents, and react to stimuli, based on their rules and internal states. Each agent concerns one entity of the real system and such agents can be heterogenous, each with their own states and rules. Currently, the proposed models in *AutoSimmune* have already covered tissue areas (upper airway and kidneys), lymphnodes, the circulatory system, bone marrow, and

[7] `http://repast.sourceforge.net/repast_simphony`

thymus, besides antigen agents, antibodies and cytokines (Tables 1 and 2; Figure 2), virus and immune system cells. Figure 2 shows detail of the *Tissue zone* with parenchymatous cells (in white), virus (in green), macrophages (in blue), PK1 substance concentration gradients (in red), dendritic cells (in star shape), blood, and lymph vessels (in cross shape).

Figure 2 – Simulation with *AutoSimmune*



Source: POSSI, 2012

Zones simulate a discrete space, divided in positions (x, y), in which agents move, where they can release cytokines or measure their surrounding concentration, eventually interacting with other agents they come in contact with. Cytokines (Tables 1 and 2) are modeled as layers superposed to the zone where they are circulating, informing the concentration of any given cytokine in each position (x, y) of the zone at a given time. Graphically, the simulation tool allows for the visualization of zones, agents, and substances in real time, as shown in Figure 2.

Table 1 – Pro-inflammatory cytokines in *AutoSimmune*

ACRONYM	BASIS SUBSTANCE	REPRESENTATION	REPRESENTED SUBSTANCES
PK1	Parenchimalkine 1	Stress factors released by tissues under damage due to infection or immune response	Heat shock proteins (HSP), uric acid and others; besides chemokines like CX3CL1, CCL3, CCL5 and CCL6
MK1	Mono-kine 1	Set of pro-inflamatory substances present in innate immune responses	IL-12, IL-8, CCL3, CCL4, CCL5, CXCL9, CXCL10, CXCL11
CK1	Cytokine 1	Set of pro-inflammatory substances present in adaptive immune responses	INF-γ, IL-2 and TNF-α
NECROSIS	Necrosis Factors	Remains of dead cells that suffered necrosis, i.e., died from a traumatic process, not by programmed cell death	

Source: Adapted from FOLCIK, AN AND OROSZ, 2007 and POSSI, 2012

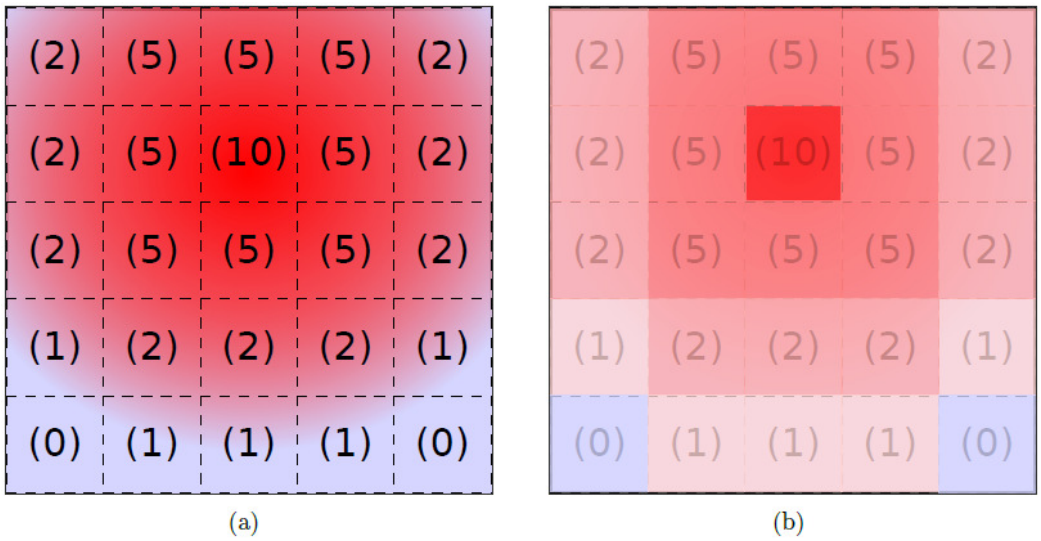
Table 2 – Anti-inflammatory cytokines in *AutoSimmune*

ACRONYM	BASIS SUBSTANCE	REPRESENTATION	REPRESENTED SUBSTANCES
MK2	Mono-kine 2	Set of anti-inflammatory substances present in innate immune responses	IL-10, CCL1, CCL17, CCL22, CCL11, CCL24, CC26
CK2	Cytokine 2	Set of anti-inflammatory substances present in adaptive immune responses	TGF-α, IL-4, IL-4, IL-5, IL-6, IL-10 and INF-γ
APOPTOSIS	Apoptotic bodies	Remains of cells that suffered programmed cell death, or apoptosis	

Source: Adapted from FOLCIK, AN AND OROSZ, 2007 and POSSI, 2012

Figure 3 illustrates how data layers simulate the diffusion of substances in the zone. In (a), continuous distribution as in the real world, and mean concentrations in each position. Notice that the liberation of this substance occurred in the position where concentration equals 10 units. In (b), the gradient discretization is presented, showing how the agents perceive the distribution of the substance.

Figure 3 – Illustration representing cytokines gradient of dispersion through layers of data



Source : Reproduced from POSSI, 2012

In addition to autoimmunity, *AutoSimmune* has also been used to investigate the role of immune response in post-infectious glomerulonephritis by *Streptococcus* pyogenes (BASTOS, 2013), the role of mast cells in controlling inflammation (SILVA et al., 2012), besides the theoretical proposition of application in the study of both *American trypanosomiasis* and *sepsis* (SIQUEIRA-BATISTA et al., 2012b).

4 THE ARTIFICIAL MACROPHAGE

After implementation of the model with the chosen *framework*, simulations were performed generating data on agents' interaction. The parameter chosen for data extraction in the simulations was the number of a certain type of agent by time interval, allowing the analysis of the agents' population increase/decrease tendency in relation to external stimuli, number of other agents, and certain moments in the process (recognition, activation, expansion, elimination, decline, and memory). These data were produced by the *framework* in electronic spreadsheet and turned into graphics. Such trend plots could then be compared to data in literature.

In *AutoSimmune*, macrophage is represented by the agent *Macrophage*, which is defined by a Java (the programming language) class with the same name. Figure 4 presents a section of *AutoSimmune* class diagram, showing briefly how the *Macrophage* class relates to the other classes in the model.

Figure 4 – Section of *AutoSimmune* class diagram, highlighting class *Macrophage*

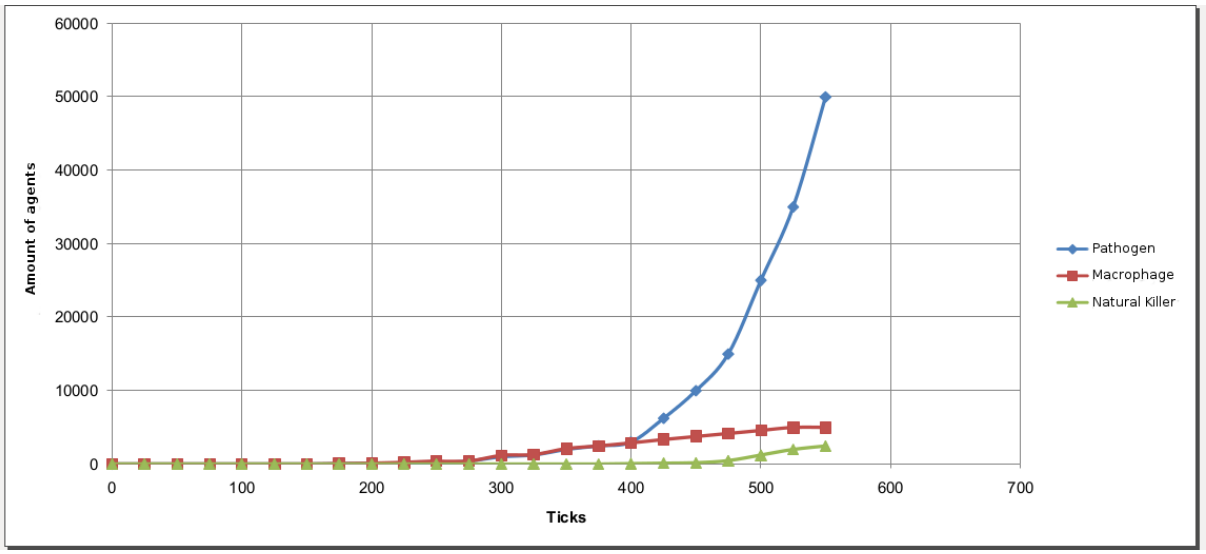
Source : Illustration developed by the authors

In *AutoSimmune*, the Macrophage agent is implemented in the *Macrophage* Java class. These agents (class instances) are inserted in the *Tissue* zone through the portals (*TissuePortal, CirculationPortal, LymphnodePortal*) when such agents detect the presence of PK1 substance (cell stress factor), CK1, MK1 (pro-inflammatory substances from innate immune response and adaptive immune response, respectively) and NECROSIS (cell remains), all of which have the role of attracting the agent, simulating diapedesis (POSSI, 2012). Once positioned in the site of infection, meeting the aforementioned substances above the limit specified in simulation parameters, *Macrophage* becomes active, producing MK1 and phagocyting antigens, dead cell debris and immune complexes, the antigens of which it may present to T helper lymphocytes, since the agent *Macrophage* extends the abstract class APC, which defines the class of cells showing antigens.

The passage of time was modeled with the concept of discrete time unit, called *tick*, offered by the *framework* (POSSI, 2012). *Tick* can be understood as the time interval needed for the environment as a whole to go from a state A to the next state B. During a *tick*, all agents, one at a time, perform their tasks, such as changing position, releasing substances, analyzing their neighborhood, etc. All actions are performed based on data from the previous tick, thus preventing the last agents to do their tasks within the same *tick* from being influenced by the actions the first ones have already taken. In the end, when the last agent in the queue has finished its actions, the *framework* moves to the next *tick*, repeating the process.

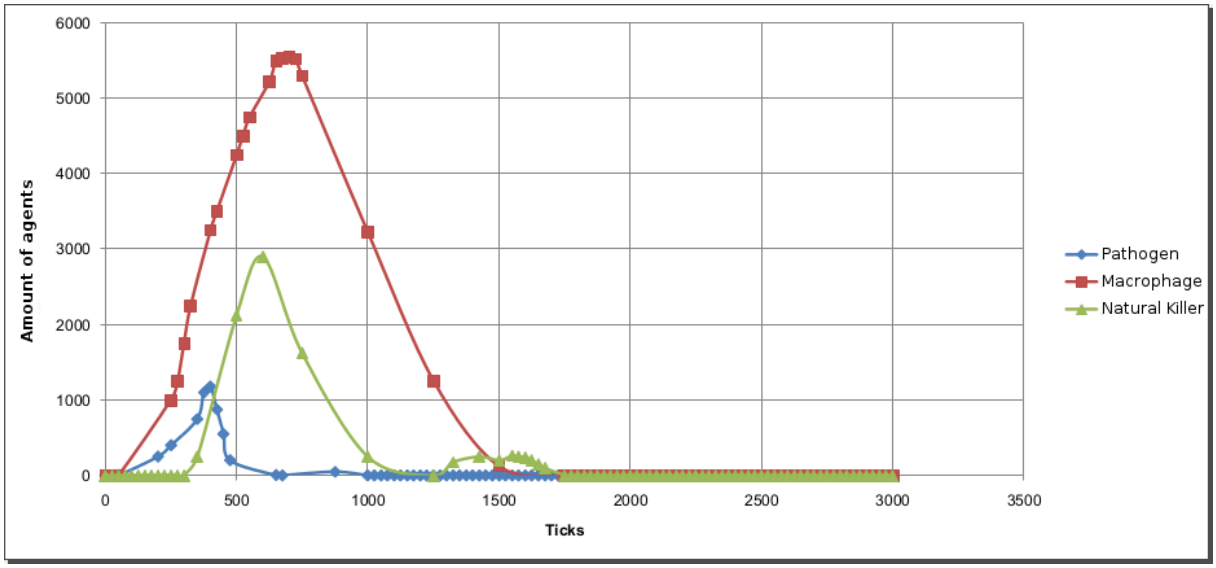
The duration of each *tick*, concerning real time, greatly varies, according to the processing power of the machine simulating the model, and has no relevance except for optimization purposes. On the other hand, the relationship between the *tick* and the real time unit (hours, days, etc.) is highly relevant to the research in question. Nonetheless, due to the scarcity of information in literature concerning duration of events, the relationship between ticks and real time units has not been modeled in *AutoSimmune* (POSSI, 2012).

Figure 5 – Innate response to an infection by a PAMP-negative pathogen



Source : POSSI ET AL., 2011

Figure 6 – Innate response to an infection by a PAMP-positive pathogen



Source : POSSI ET AL., 2011

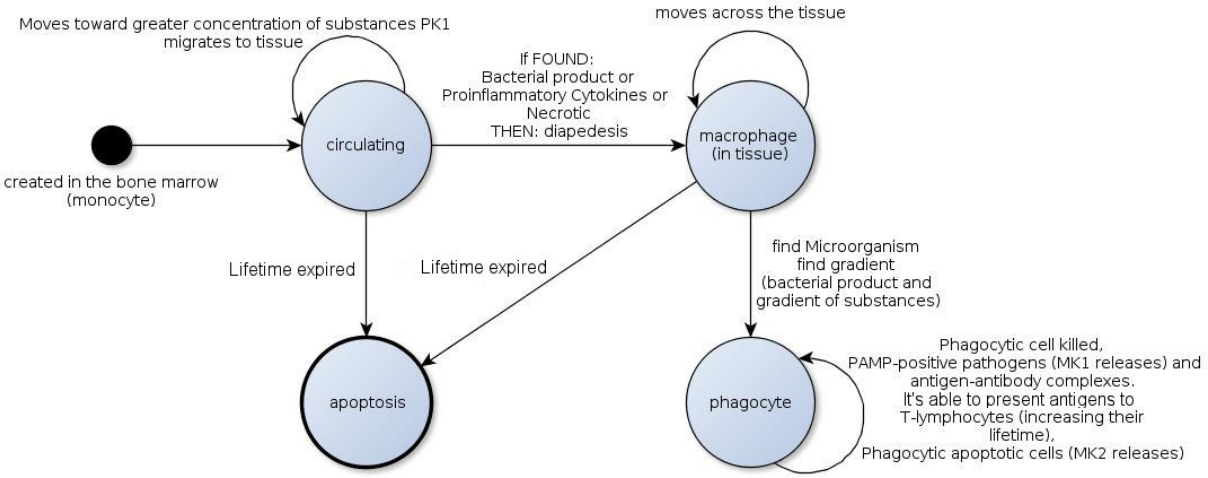
Figures 5 and 6 show the first experiments conducted with *AutoSimmune*, evidencing the behavior of agents *Macrophage*, consistent with the literature in the PAMP-dependent (PAMP = pathogen-associated molecular patterns) primary response to infectious agents. In Figure 5, the infectious agent did not have a PAMP antigen pattern recognizable by agent, *Macrophage*. Thus, the latter was not activated. In Figure 6, with a PAMP-positive pathogen, *Macrophage* was activated, releasing cytokine MK1 to recruit more agents to the site, increasing phagocytosis, eliminating infection and cleaning the site to facilitate tissue repair (POSSI et al., 2011). The adaptive response in these experiments were disabled.

Despite presenting a coherent behavior, agent *Macrophage* does not possess the rules and states needed to model the immune response regulation, essential for the simulation of sepsis, since *AutoSimmune* is based solely on the hypothesis that the decline of immunological response happens due to the absence of antigens to stimulate it (POSSI, 2012). Thereof, it becomes

necessary to extend agent *Macrophage*, including state M2 (anti-inflammatory *Macrophage*) and the production of the substance that represents anti-inflammatory cytokines, MK2.

Figure 7 shows the state machine describing the rules of the agent that represents the new model proposed for *Macrophage*. It illustrates what has been implemented as an extension of *AutoSimmune* to simulate this kind of cell related to the immune response in the occurrence of diseases such as sepsis.

Figure 7 – Rules of agent *Macrophage* in the simulation of sepsis



Source : Illustration developed by the authors

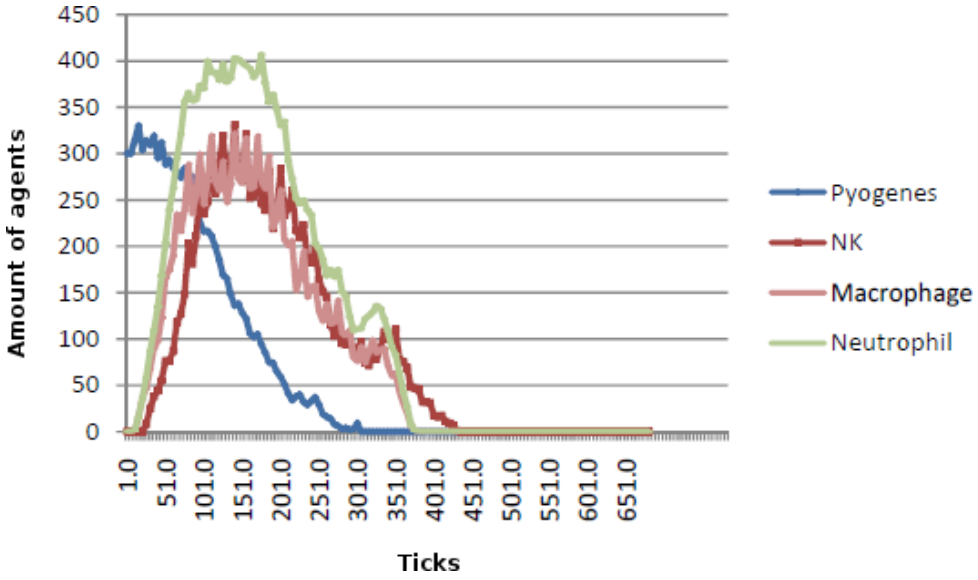
In the tissue state, the agent moves from circulating state (monocyte) to *Macrophage* state, where it identifies and phagocytes microorganisms, taking into account the molecular affinity – represented by a string of contiguous bits present in each agent (BASTOS, 2013). In the phagocyte state, when *Macrophage* phagocytes necrotic cells or bacteria, it produces pro-inflammatory cytokine (MK1). On the other hand, when it phagocytes apoptotic cells (other macrophages or neutrophils), it produces the anti-inflammatory substance (MK2).

When its lifetime ends, the agent *Macrophage* alters its state into cell death, which includes apoptosis cases, simulating what happens in the organism of *Homo sapiens sapiens* (BISWAS et al., 2012).

Recent studies with the use of *AutoSimmune* have generated initial data, mainly concerning the role of macrophage. Thus, Bastos (2013) was able to demonstrate the role of macrophage in the immunopathogenesis of post-streptococcal glomerulonephritis (PSGN), a disease that affects human beings, characterized by proliferative lesions in renal glomeruli as a consequence of infection in the upper airway or skin by the infectious agent S. pyogenes (BASTOS, 2013).

Initially, for making possible PSGN simulation, the amount of bacteria needed for the infection to occur in the airway was identified (*AirWayTissue*). Once this was done, innate immune response was activated, with the direct action of natural phagocytes, including macrophages (Figure 8), which have the role of starting to phagocyte bacteria and necrotic cells, besides sending inflammatory signals to the other agents.

Figure 8 – Bacterial infection in the upper airway, with the presence of macrophages



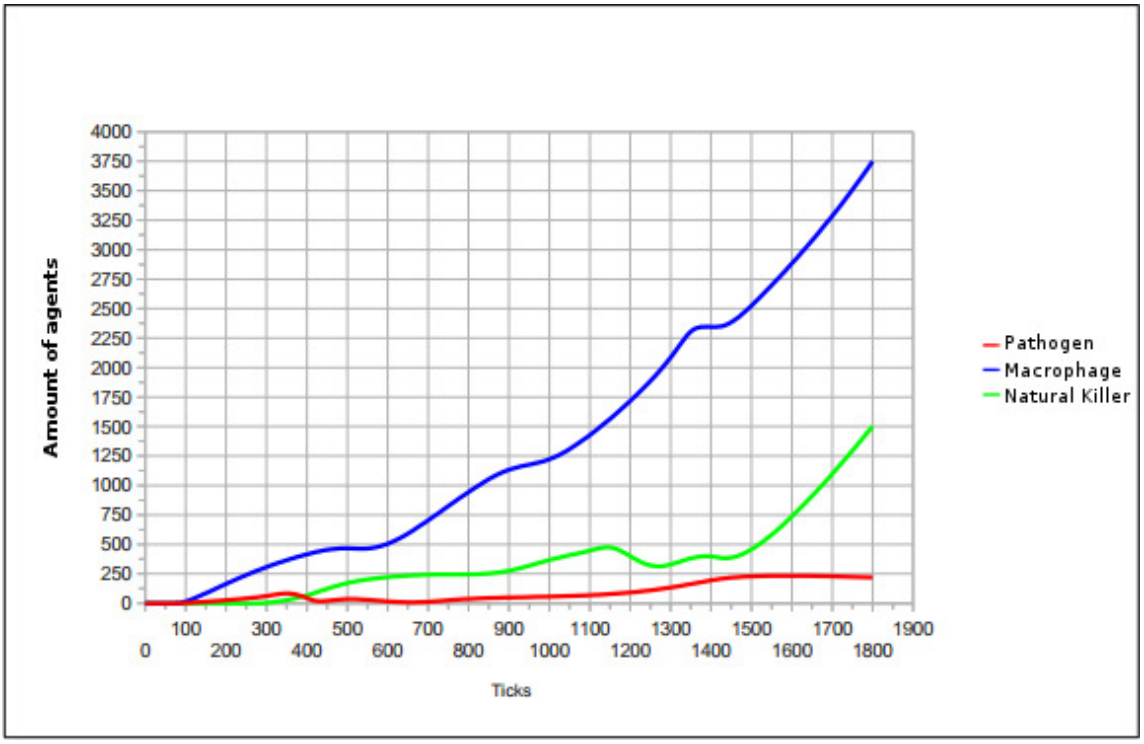
Source: BASTOS, 2013

After activation of the adaptive immune response, antibodies derived from B cells are directed to the bloodstream and, according to their specificity, bind to pathogenic bacteria, originating immunocomplexes. Nonetheless, when filtered by the kidney, immunocomplexes are trapped in the glomeruli, prompting neutrophils and macrophages to start inflammation which results in glomerular lesion, triggering PSGN (BASTOS et al., 2014; SIQUEIRA-BATISTA et al., 2015).

A significant aspect described by Possi (2012) concerns the role of the interaction between macrophages and NK cells in the elimination of pathogens, which were modeled with the following characteristics: (1) Infecting the cells; (2) having an incubation period; (3) reproducing and killing the cell after leaving it. Thereby, in the first simulation, there were NK cells for them to be able to develop their role during infection (Figure 9).

Notice in Figure 9 that macrophage cells have started to answer the infectious process recruiting NK cells, which were able to control the replication of the agent – a parallel of this would be the reduction in viral load (red curve) – when compared to the increase in NK cell numbers (green curve) (POSSI et al., 2011). However, the innate immune response was not competent enough to completely eliminate the virus. Janeway, Travers and Walport (2001) achieved similar results in their study, insofar as NK cells only controlled viral replication, being unable to eliminate it. Additionally to their study, Janeway, Travers and Walport (2001) concluded that for achieving total elimination, it is necessary to subsequently activate cytotoxic T lymphocytes (CTL). Thereby, Possi et al. (2011) consider the result of the simulation to be positive, confirming that the model presented the expected behavior regarding the biological domain.

Figure 9 – Course of infection with NK action



Source: POSSI ET AL., 2011

5 CONCLUSION

The immune system computational investigation may give rise to new hypothesis – besides allowing the conduct of preliminary tests before experimenting *in vitro* and *in vivo* – contributing with new knowledge over the pathophysiology of human diseases. In this context, the investigations *in silico* about the role of macrophage in sepsis – the proposition of *artificial macrophage*, the first outlines of which were presented in this brief communication – will permit the development of more accurate and conclusive analyses and experiments. For such, the decisive steps will be (i) the development of studies in the area of immunology and pathogenesis of infectious diseases and (ii) the improvement of the model – *AutoSimmune* – in computational terms, what may also bring benefits to the computer area concerning bio-inspired systems.

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