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Pro-Inflammatory Cytokines in Sepsis: Biological Studies and Prospects From *In Silico* Research

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Abstract

Sepsis is one of the leading causes of death in intensive care units (ICUs) and is responsible for thousands of annual deaths worldwide. The pro-inflammatory cytokines are necessary for the control of infection and are the primary focus of this paper. Due to their central role in the pathogenesis of sepsis, more emphasis is needed on the use of cytokine as biomarkers. Implementation of the cytokines in the *AutoSimmune* for immune system simulations may improve understanding of aspects of the physiopathology of disease in humans. We present the principal aspects of the pathogenesis of the pro-inflammatory response in sepsis and the possibilities of their modulation in order to alter the course of this illness. We highlight the main pro-inflammatory cytokines that may be used as biomarkers in clinical practice. We also discuss the perspectives of sepsis *in silico* investigation, using the *AutoSimmune* computational system. Sepsis remains a true challenge in contemporary clinical practice, especially in terms of diagnosis, therapeutics, and prognosis. A greater understanding of inflammation in sepsis – especially in relation to cellular and molecular participation in the development of the morbid process – has the potentiality for the development of new investigative methods and outcome prediction, elements that may aid in offering good patient care.

Keywords: Cytokines; Inflammation; *In silico* investigation; Sepsis

Introduction

Sepsis is a common cause for admission and fatal outcomes in intensive care units (ICU) [1]. Most of these hospital-acquired infections occur in infirmaries and ICUs [2], however, many of the patients requiring intensive treatment, acquire them outside of the hospital environment [3]. The cost of septic patients in the United States is $14 billion per year [4]. Therefore, from the clinical and public health perspective it is an extremely important condition, not only because of its high incidence, but also because of its mortality [5,6].

The diseases whose different infectious processes (bacterial, viral, fungal or parasitic) [3] may culminate in the development of sepsis, which is a life-threatening organ dysfunction caused by a dysregulated host response to infection [7]. The key mediators and regulators in sepsis are the cytokines, a group of endogenous inflammatory mediators and immunomodulatory proteins. They are generally divided into two groups, anti- and pro-inflammatory [1], responsible for coordinating the host defense mechanisms against pathogens [8].

Pro-inflammatory cytokine release leads to activation of the innate immune response, characterized by the production of other effector or immunoregulatory cytokines [8]. Generalized participation of pathogen-responsive cells that results in sepsis occurs simultaneously with the production and secretion of a number of pro-inflammatory cytokines. In this context, the interleukins 1 (IL-1), 2 (IL-2), 6 (IL-6), 8 (IL-8), 12 (IL-12), TNF-α, are considered essential for its evolution [9].

In conjunction with intense pro-inflammatory activity, anti-inflammatory cytokines are also produced – interleukins 4 (IL-4), 5 (IL-5), 10 (IL-10), 11 (IL-11), and 13 (IL-13), which are implicated in the development of anergy and the slowing of responses to etiologic agents, generating a compensatory anti-inflammatory response syndrome (CARS) [10]. This anti-inflammatory response is more expressive in situations in which the patient survives the disorders related to systemic inflammation [11]. However, the balance between pro- and anti-inflammatory mediators is a dynamic process and in some cases a situation of intense "immunological disharmony" may occur, known as MARS (mixed antagonist inflammatory response syndrome) [12].

Regulation of the pro-/anti-inflammatory equilibrium is carried out by a complex network of mediators, and is associated with the progression of sepsis, whether it comes to resolution or to fatal outcome. Biological molecules like the cytokines – as well as hundreds of other cellular elements – are identified in several studies as potential biomarkers, useful in recognition, prediction of progression, and treatment of infection and sepsis [13].

Cytokines are central in the pathogenesis of sepsis and for this reason more emphasis should be given to their use as biomarkers [14], useful in disease diagnosis and consequently reducing lethality risk, important factors in clinical practice [15,16]. The most used marker is the lactate, whose levels increase in sepsis, however it is believed that the pro-inflammatory cytokines may improve its efficacy [17].

The implementation of the cytokines in *AutoSimmune* (immune system simulator) is performed as values scattered in arrays of data parallel to the agent interaction environment [18]. In this paper, we present the principal aspects of the pathogenesis of the pro-inflammatory response in sepsis and the chance of its modulation to change the disease course. We, also, highlight the main pro-

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inflammatory cytokines, which can be used like biomarkers in the clinical setting. Finally, we argue about the new perspectives for *in silico* investigation of sepsis, using the *AutoSimmune* computer system.

Methods

The literature review consisted of consultations to the LILACS (*Literatura Latino-Americana e do Caribe em Ciências da Saúde*), SciElo Brasil (Scientific Eletronic Library Online), Medline, and CAPES databases. The terms "sepsis" and "cytokines" were defined based on DeCS (Descritores em Ciências da Saúde). The research, performed on February 18, 2016 yielded 606 citations, published within the last 10 years, in English, Spanish and Portuguese. Of these, 24 papers referencing the interface between sepsis and pro-inflammatory cytokines were selected.

Paper selection occurred as follows: pre-selection upon reading the title and abstract, and subsequently, evaluation of the papers initially selected, with later exclusion of paper not related to human's diseases and those that, after reading the entire paper, were not associated with sepsis and pro-inflammatory cytokines (Table 1).

The information was systematized and organized into two sections: (1) *The pro-inflammatory response and its mediators*, and (2) *The cytokines as biomarkers to sepsis*. The third section – *AutoSimmune: general characteristics and pro-inflammatory cytokines* – provides information about the computer tool that had developed for *in silico* investigation of the immune system. This tool has been used for the study of sepsis, focusing on the attributes of the cytokines that participate in the physio-pathological process.

The pro-inflammatory response and its mediators

Actually, sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection [7]. For clinical operationalization, the organ dysfunction can be represented by an increase in the Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score of 2 points or more, which is associated with in-hospital mortality greater than 10%. Septic shock should be defined as a subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone [19].

In sepsis, the clinical manifestations, the morbidity, and the risk of fatality are influenced by specific virulence factors – endotoxins, exotoxins, and by the species of pathogen involved in the infection [20]. These influences are attributed to the cytokine profiles, and the distinct fatality rates observed in sepsis result from infections caused by gram-negative or gram-positive bacteria or other infectious agents in humans. Additionally, it is possible that anti-mediator therapies have different actions depending on the species or group of infectious agent, which makes identification and knowledge of the agent even more important [20].

With regards to the pro-inflammatory response, the PRR (pattern recognition receptors) – such as the Toll-like and NLRs (nucleotide-binding oligomerization domain receptors) are essential in the recognition of PAMPs (pathogen-associated molecular patterns). They are also able to recognize DAMPs (damage-associated molecular pattern molecules), endogenous molecules related to tissue damage. These molecules are released by different cells when affected by different types of stress and by necrotic factors, and are able to activate immune system receptors in a manner similar to the activation induced by bacterial patterns. Examples include the release of large quantities of fibrinogen, hyaluronic acid, and the high-mobility group box-1 protein (HMGB-1) during the inflammatory process, molecules that are capable of amplifying the pro-inflammatory response event more (Figure 1) [9,14,21].

The physiopathology of sepsis is associated with distinct mediators, all of them have pleiotropic effects, although they differ in terms of source, kinetics, and stage of sepsis in which they are predominant. Thus, they are able to carry out different functions, connecting various immune response pathways (Table 2) [14,22]. This inflammatory response, however, must be strictly controlled: it requires protection from the causative microorganism, but at the same time, an exaggerated activation of NLR induces caspase-1 activity, which leads to intense processing of pro-inflammatory cytokines such as IL-1 and IL-18, resulting in promotion of significant damage to the organism [23,24].

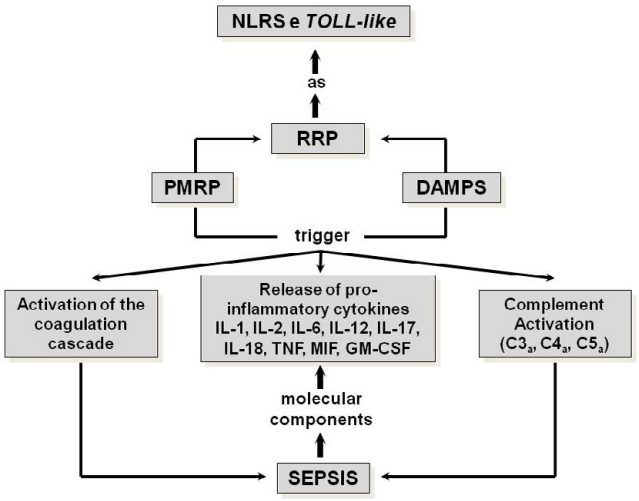

Figure 1: Molecular mechanisms in SEPSIS.

PAMPS: Pathogen-Associated Molecular Patterns; DAMP: Damage-Associated Molecular Patterns; PRRs: Pattern Recognition Receptors; NLRS: Nucleotide-Binding Oligomerization Domain Receptors; HMBG-1: High Mobility Group Box-1 Protein

Source: Elaborated by the authors based on references [9,23,27].

Sources used	Key words searched	Number of citations	Number of publications selected
MEDLINE	Sepsis+cytokines Limits: humans, full-text, free access	268	16
SCIELO	Sepsis+cytokines Sepsis+cytokines	16	1
LILACS	Sepsis+cytokines Sepsis+cytokines	54	3
Capes database	Sepsis+cytokines Sepsis+cytokines	258	4
Total selected publications			24

Table 1: Distribution of publications obtained in the LILACS, MEDLINE, SCIELO and Capes databases.

Cytokines	Secreted By	Sites of Action and Biological Effects
Interleukin-1α (IL-1α)	Macrophages, dendritic cells, fibroblasts, endothelial cells, keratinocytes, hepatocytes.	Endothelial cells: activation (inflammation, coagulation) Hypothalamus: fever Liver: synthesis of acute phase proteins
Interleukin-1β (IL-1β)	Macrophages, dendritic cells, fibroblasts, endothelial cells, keratinocytes, hepatocytes.	Endothelial cells: activation (inflammation, coagulation) Hypothalamus: fever Liver: synthesis of acute phase proteins
Interleukin-2 (IL-2)	T cells	T cells: proliferation and differentiation to effector and memory cells; promotes development, survival and function of regulatory T cells NK cells: proliferation, activation B cells: proliferation, antibody synthesis (*in vitro*)
Interleukin-6 (IL-6)	Macrophages, endothelial cells, T cells	Liver: synthesis of acute phase proteins B cells: proliferation of antibody-producing cells
Interleukin-12 (IL-12)	Macrophages, dendritic cells	T cells: Th1 differentiation NK cells and T cells: IFN-γ synthesis, increased cytotoxic activity
Interleukin-17A (IL-17 A) **Interleukin-17F (IL-17 F)**	T cells	Endothelial cells: increased chemokine production Macrophages: increased chemokine and cytokine production Epithelial cells: GM-CSF and G-CSF production
Interleukin-18 (IL-18)	Monocytes, macrophages, dendritic cells, Kupffer cells, keratinocytes, chondrocytes, fibroblasts, osteoblasts.	NK cells and T cells: IFN-γ synthesis Monocytes: GM-CSF, TNF, IL-1β expression Neutrophils: activation, cytokine release
Tumor necrosis factor (TNFα, TNFβ)	Macrophages, NK cells, T cells	Endothelial cells: activation (inflammation, coagulation) Neutrophils: activation Hypothalamus: fever Liver: synthesis of acute phase proteins Muscles, fat: catabolism (cachexia)
MIF (microphage migration inhibitory factor)	Expressed by T and B lymphocytes, macrophages e monocytes.	Regulates the innate immune response through TRL4 modulation
G-CSF (granulocyte colony-stimulating factor)	Activated T cells, macrophages and endothelial cells.	Bone marrow: increased neutrophil production
M-CSF (monocyte colony-stimulating factor)	Activated T cells, macrophages, endothelial cells and fibroblasts.	Bone marrow: increased monocyte production
HMGB-1	Activated immune cells (macrophages, monocytes, dendritic cells).	Interaction with TRL2 and TRL4

Source: Elaborated by the authors based on references [22,23,27].

Table 2: Principal pro-inflammatory cytokines, their sources, sites of action and their biological functions.

In fact, the role of the pro-inflammatory cytokines in sepsis is crucial [21,25], given that they regulate the immune response to infections and are fundamental in regulating inflammations and traumas [26]. Then, TNF-α and IL-1 play important roles, since they bind to target cells and induce secretion of more inflammatory mediators [21,25].

The cytokine TNF-α is involved in the innate immune response, especially in macrophages and lymphocytes, in differentiation and inactivation of immune cells, and in apoptosis. In interaction with other cytokines, it promotes the release of pro-inflammatory effector molecules. IL-1 is responsible for the induction of fever and coagulation and for the releasing of inflammatory cells [8]. Other cytokines involved in sepsis are IL-6, IL-8, IL-11, IL-12, IL-17, IL-18, IL-20, IL-33, MIF, and IFN-γ. Aside from these, several other chemokines also attract inflammatory cells [26].

The IL-17 family of cytokines has emerged as important mediator of immune regulation, since it cooperates with the pro-inflammatory response by triggering the production of other cytokines such as IL-1β, IL-6 and TNF-α, in addition to signaling between lymphocytes and phagocytes [18,27,28]. Additionally in patients with severe sepsis, high levels of IL-1α, IL-6, IL-8, monocyte chemotactic protein-1 (MCP-1) and granulocyte colony-stimulating factor (G-CSF) are associated with lethality [29].

The primary function of IL-8 is induction of chemotaxis in target cells, including neutrophils, functioning as a chemical signal that draws them to the site of inflammation. IL-18, produced by macrophages and other cell types, is considered important in the physiopathology of sepsis [26], on account of the high levels detected among patients presenting this condition [30]. Attention has been given to IL-33 due to increased plasma levels among septic patients, 3 hours after admission to the ICU. In these patients, the positive correlation with pro-calcitonin (PCT) levels was considered important to the diagnosis of sepsis and was associated with disease severity [31].

One of the relevant aspects of the pro-inflammatory state in sepsis is the activation of the complement system in the early stages of sepsis, generating large quantities of C3a and C5a. At high concentrations, these molecules have several deleterious effects, since they induce vasodilation, increase vascular permeability, potentiate platelet aggregation and neutrophil activation/aggregation. These events are responsible, in part, for the microvascular alterations present in septic shock. However, the complement system also modulates responses associated to TLR4 binding and cytokine release, such as macrophage migration inhibitory factor (MIF) and HMGB (High-Mobility Group Box 1 Protein) [14] (Figure 2).

The TLR receptor family was initially discovered in *Drosophila*

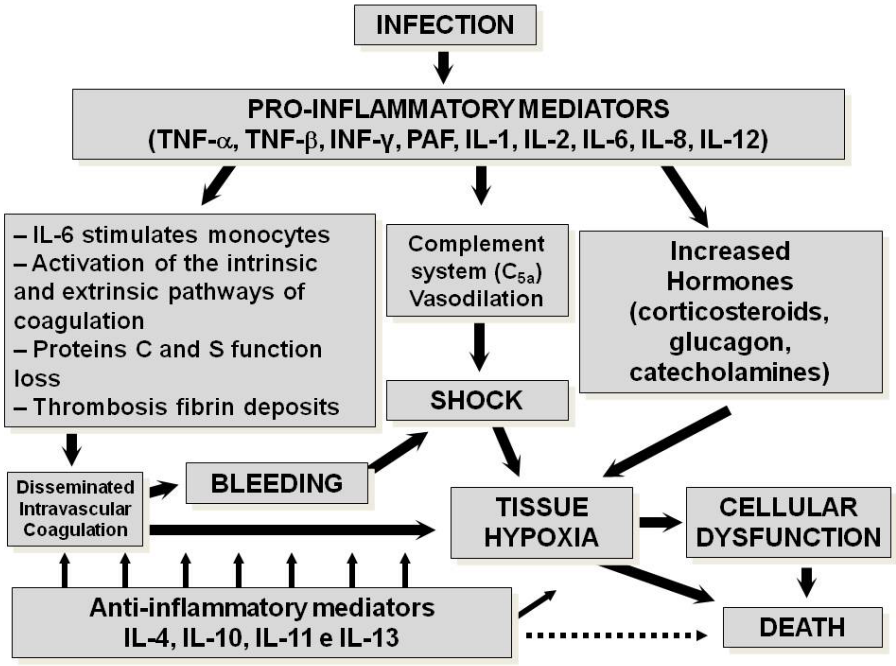

Figure 2: The host recognizes the pathogen during infection. This recognition leads to recruitment and a pro-inflammatory cytokine response (TNF- α, TNF-β, INF-γ, PAF, IL-1, IL-2, IL-6, IL-8, IL-12 and IL-6). The pathogen can proliferate and trigger hypercytokinemia, leading to tissue damage and even death of the host.

melanogaster and is very important in recognition of microbial pathogens. On the extracellular surface, receptors include TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10, while TLR3, TLR7, TLR8 and TLR9 are located in the endoplasmic reticulum and the endosome. The most investigated members of the TLR family are TLR-2 and TLR-4, since they demonstrate effective binding to microbial ligands, especially endotoxins or lipopolysaccharides (LPS) of gram-negative bacteria [32]. TLR-5 recognizes flagellin, present in the flagellum of prokaryotes, while TLR-9 recognizes viral DNA and hemozoin in Plasmodium. In summary, expression of TLR receptors in various cells involved in the immune response is not static; rather it is rapidly modulated in response to pathological agents, cytokines, and environmental stress [33]. The subsequent binding of TLR components to specific components of the microbial cell stimulates intracellular signaling, which triggers an increase in transcription of pro-inflammatory molecules such as TNF-α and IL-1β [34]. Bacteria and virus have developed strategies to escape the immune response, which, consequently, promote their growth, survival and replication. In one of these strategies, the pathogen becomes invisible to the immune system, which, unable to unleash an effective response. The other is the pathogen's ability to stimulate the immune system, a strategic known as cytokine storm. In this way, due to failure of the infection, the tissue is damaged [35].

Antibiotics are generally regarded as the best and most widely used strategy for treatment of bacterial infections. However, the indiscriminate and inappropriate of these drugs has resulted in multidrug-resistant (MDR) bacteria [35]. However, the use of combination therapy between antibiotics bring good perspective in the treatment of sepsis, due to reduction in levels of IL-6 in blood plasma, but not TNF-α [36]. There are prospects of approach on this subject using the *AutoSimmune*. MIF is also a pro-inflammatory cytokine secreted by leukocytes. MIF release is strongly induced by microbial products (endo- and exotoxins) and pro-inflammatory mediators, such as TNF, IFN-γ and C5a. The molecule is able to connect the immune system with the endocrine system in response to stress. It is also secreted by the hypothalamus, anterior pituitary and the adrenals [23]. In contrast to other cytokines, MIF is constitutively expressed and stored intracellularly. After its secretion, it functions as a classic pro-inflammatory cytokine, since it promotes innate and adaptive immune responses through macrophage and T cell activation [22]. Curiously, the pro-inflammatory actions of MIF are mediated by tautomerase, an enzyme coded by a domain containing an evolutionarily conserved catalytic site. In addition to its pro-inflammatory effects, MIF also (i) induces and amplifies the production of other pro-inflammatory cytokines and (ii) positively regulates TLR4 expression by phagocytes. At high concentrations, it prevents macrophage apoptosis, leading to a sustained inflammatory response [25-27].

HMGB is the focus of a great deal of research in the context of sepsis, because its function as a late mediator, that can be verified in the most patients with this condition. It is secreted by cells activated by the immune system interacting with TLR-2 and TLR-4, generating inflammatory responses that are similar to those initiated by LPS. This induction also triggers activation of coagulation and neutrophil recruitment [27].

The role of the myeloid related proteins, the most abundant cytoplasmic proteins of the neutrophils Mrp08 (S100A8) and Mr14 (S100A9) is also worth mentioning. They can form heterodimers, that are released in response to stress and their direct antimicrobial effect is commonly associated with phagocytosis [37]. The Mrp8/14 complexes amplify the phagocytic inflammatory response triggered by endotoxin, increasing recruitment of inflammatory cells to lesion sites. Mrp8 also acts as an active component of the binding of the Mrp8/14 complex to TLR-4, increasing TNF-α expression [9,23,38]. Additionally, it promotes the systemic inflammatory response by activation of the TLRs [38] and Mrp8 and Mrp14 levels are elevated in patients with sepsis [37].

The cytokines as biomarkers in sepsis

It is increasingly recognized that the inflammatory response and unregulated production of cytokines play fundamental roles in the development of organs dysfunction. The emphasis on the possibility of using the cytokines in medical practice is due precisely to the central role they play in the pathogenesis of sepsis [14].

The research for biomarkers of sepsis resulted in the investigation of several molecules, including pro-calcitonin (PCT), C-reactive protein, and the interleukins, which include IL-1, IL-6, IL-8, and TNF [9,29]. Some studies also include IL-1β, IL-6, IL-8, MCP-1, and G-CSF, as cytokines positively correlated with the progression to MODS. In terms of predicting fatality, IL-1β, IL-6, IL-8, MCP-1, and G-CSF are more strongly related to the possibility of early death (<48 h), while IL-8 and MCP-1 are more predictive of later fatality [14, 23].

MIF is elevated in sepsis and septic shock, and has been correlated with mortality prediction in ICU infections [14,23,39]. During sepsis progression to septic shock there is a significant increase of sTREM-1 (soluble triggering receptor expressed on myeloid cells-1) [28]. High Mobility Group B-1 protein (HMGB-1) appears to be a late mediator in sepsis, and has been shown to be a marker of severity [23,24].

To date, however, cytokine patterns associated with septic shock and sepsis as well as other developments – favorable or not – are far from adequately determined. It is important to highlight, however, that in terms of prediction of results, no biomarker is 100% precise. This it is extremely important to combine biomarker levels with severity scores in order to produce the best results [14].

Recently, markers such as interleukins (IL-6, IL-8 and IL-10), has been used in diabetic patients due to obtaining levels changed in blood plasma. However, those other markers such as TNF-α, IFN-γ, IL-β1 and IL-13 were not detected or were at very low levels [40]. Other markers such as microRNAs (which do not encode proteins, but regulate gene expression by inhibiting translation and transcription of mRNA) had also been used in sepsis. One of these microRNAs, miR-133a, was proposed to play an essential role in the development of cancer and inflammation [41]. These recent approaches provide new perspectives for use in *AutoSimune*.

AutoSimmune: Overall characteristics and pro-inflammatory cytokines

Simulations of the immune system have been used in the study of certain clinical situations in order to better understand the pathophysiological aspects of disease in humans [42,43]. In this sense the use of multi-agent systems is interesting, in which cells of the organism, their products (e.g. cytokines, antibodies, etc.) and the antigens can be implemented as independent agents that interact amongst themselves, following a specific set of rules for each of these agents, for different contexts [42,44].

AutoSimmune is an immune system simulator that had developed at Universidade Federal de Viçosa, using the Repast Simphony framework, originally proposed by Possi [45] for the study of autoimmunity. In this model, we adopted as agent any component that participates in the immune response, such as antigents (recognized as not-*self*), antibodies, and cells [42,43].

Adaptation of *in silico* research for sepsis via the *AutoSimmune* is performed observing the granularity level of the simulator, focusing the analyses on the cellular level and from there, proposing changes to the model. Subsequently, in addition to insertion of the neutrophil agent and adaptation of the macrophage, insertion of the pro-inflammatory substances IL-1, IL-2, TNF-α and others is performed, as is creation of the pathogenic agent. In this way the association with the rest of the implemented model allows for simulation of the pro-inflammatory mechanisms and the provision of the indicators of sepsis [42].

The cytokines and other substances are implemented in *AutoSimmune*, as values dispersed in data matrices parallel to the interaction environment of the agents, whereby each agent can perceive the concentration of each of these substances at a given moment, for its position and surroundings. There are two abstract groups of cytokines in the system, based on their functionality. The first is represented by pro-inflammatory cytokines, which amplify inflammatory responses, and the second is made up of anti-inflammatory cytokines which suppress the inflammatory response [18].

The pro-inflammatory substances included in the model are: (1) PK1: stress factors released by tissues undergoing damage as a result of infection or immune response: heat-shock proteins (HSP), uric acid, and others; in addition to chemokines such as CX3CL1, CCL3, CCL5, CCL6; (2) MK1: a set of pro-inflammatory substances present in innate immune responses; including the substances IL-12, IL-8, CCL3, CCL4, CCL5, CXCL9, CXCL10 and CXCL11; (3) CK1: group of pro-inflammatory substances present in adaptive immune responses: IFN-γ, IL-2 and TNF-β; and (4) NECROSIS: fragments from cells that have undergone necrosis, in other words, that have died in a traumatic process and not by programmed cell death [15,46].

The anti-inflammatory substances included in the model are: (1) MK2: A group of anti-inflammatory substances present in innate immune responses; including the substances IL-10, CCL1, CCL17, CCL22, CCL11, CCL24 and CCL26; (2) CK2: a group of anti-inflammatory substances present in adaptive immune responses; represented by the substances TGF-β, IL-4, IL-5, IL-6, IL-10, IL-13 and IFN-γ; and (3) APOPTOSIS: fragments from cells that have undergone programmed cellular death or apoptosis [42,45].

Studies using *AutoSimmune* are currently under development, with this configuration, with some preliminary results published [25,42,43]. At the moment, the group is working on breaking down the two large abstract groups of cytokines in order to substitute groups CK1 and CK2 for individual implementation of IL-1, IL-2, IL-4, IL-10, IFN-γ and TNF-α. This way, each class of agent must be modified in order to recognize and correctly interact with each of these new substances. In this way we will increase the granulosity and complexity of the system, which will result in greater computational cost [45]. It is important to point out that in the coming years, a combined data-driven and mechanistic approach will likely be a study area in further expansion [47].

With these modifications, we hope to obtain simulation results more faithful to those presented in the literature. From there it will be possible to experiment with simulations in which levels of a given substance can be altering to look for a possible physio-pathological events – and diagnostic markers – for sepsis.

In silico experimentation can result in the elaboration and validation of new hypotheses and knowledge, which, in conjunction with other analysis and approaches, such as those involving proteomics and transcriptomics, may provide important tools for understanding the mechanisms related to sepsis. As a result, solutions or hypotheses could be tested prior to *in vitro* and *in vivo* experimentation. The complexity of the simulator increases as it is extended, which will certainly require greater computational power. However, this computational power continues to expand and its best usage is guaranteed by techniques such as parallel programming. More conclusive and calculated

experimentation and analysis will be possible with the natural improvement of the *AutoSimmune* model [42]. Specifically for sepsis, the computer model is potentially significant with respect to prediction of possible pathogenesis, according to the physiological conditions of the host. Additionally, there is the possibility of exploring the numerous combinations of cytokines, and also the provision of a biological view of immunity [48]. We hope that the *in silico* approach will allow for a better understanding of the mechanisms that enable perspectives that promote advances in the identification of sepsis biomarkers, with the goal of earlier sepsis diagnosis.

Final Considerations

The incidence of sepsis and the consequent fatality risk has increased over the past years, especially in the United States [32] and the condition is considered the leading cause of death in intensive care units [1].

The established interaction between the pathogen and the host modulates the development of sepsis. When transferred to host cell surface receptors (monocytes, macrophages, dendritic cells, and neutrophils), and upon recognition, endotoxins and proteins of microbial origin determine several cellular activation events and cytokine production, resulting in Sepsis [42].

The pathophysiology and diagnosis of sepsis, its management and prognostic prediction are in fact, great challenges [8]. Most decisions involving high-risk patients are based on clinical and laboratory data that often have reduced precision. In this sense, a broader knowledge of systemic inflammation is needed, including those involving in silico research. Similarly, identification of new technologies for sepsis detection at earlier stages and patient stratification are extremely important and necessary [42].

In terms of human sepsis, future studies should consider patient heterogeneity, as well as the types of infections included in the tests ordered and the predominant immune response phenotype [37]. It is hoped that the complexity involved in the various interactions between pathogen and host cells and their products in the development of sepsis [42] may be discovered and better understood by application of computational modelling [47]. Additionally, use of biomarkers in the diagnosis of the disease may allow for early intervention [17], direct choice of antibiotic therapy [15], and reduce consequent risk of fatality [17].

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