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$CD4^{+}CD25^{+}$ T lymphocytes and regulation of the immune system: perspectives for a pathophysiological understanding of sepsis

Linfócitos T CD4+CD25+ e a regulação do sistema imunológico: perspectivas para o entendimento fisiopatológico da sepse

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ABSTRACT

The systemic inflammatory response represents the core pathogenic event of sepsis, underlying clinical manifestations and laboratory findings in patients. Numerous studies have shown that CD4+CD25+ T lymphocytes, also known as regulatory T lymphocytes (Treg), participate in the development of sepsis due to their ability to suppress the immune response. The present article discusses the role of Treg lymphocytes in sepsis based on a specific search strategy (Latin American and Caribbean Health Sciences / Literatura Latino-americana e do Caribe em Ciências da Saúde - LILACS, PubMed, and Scientific Electronic Library Online - SciELO) focusing on two main topics: the participation of Treg cells in inflammation and immunity as well as perspectives in the computational physiological investigation of sepsis.

Keywords: Regulatory T lymphocytes; Sepsis/pathophysiology; Sepsis/therapy; Inflammation; Immunity; Computer simulation

INTRODUCTION

The incidence of sepsis has increased dramatically during the past two decades. It is estimated that 1.5 million people in the United States and 1.5 million in Europe develop severe sepsis and/or septic shock each year, 35% to 50% of whom die.[1] This large number of fatal cases has triggered a broad range of studies aimed at understanding the intricate pathogenic mechanisms of sepsis in association with the development of immunomodulatory therapy.[1-3]

In patients with sepsis, the systemic inflammatory response is disorganized due to the disruption of the complex balance between pro- and anti-inflammatory mechanisms.[4] Many components of the human immune system are involved in anergy and reducing of the response to microorganisms, which characterize typical immunosuppression and may be designated as Compensatory Anti-inflammatory Response Syndrome (CARS).[5] In this regard, some T lymphocyte populations, that have been described with increasing details in the literature, seem to evade thymic selection or undergo a process of "thymic education" in which they acquire a status different from that of so-called "traditional" lymphocytes. For example, *self-reactive* lymphocytes, which mature exclusively in the thymus, play a prominent role in the regulation of autoimmunity. These cells are called CD4+CD25+ regulatory T lymphocytes (Treg).[6]

Treg (CD4+CD25+) cells play an important role in immune regulation.

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Notice that the control of the adaptive immune response is critical for the operation of immune system. Among the functions of the immune response that closely depend on such regulation, the responses signaled by self-reactivity are of great importance and are crucial to internal medicine with respect to autoimmune[7] and infectious diseases. Several studies have elucidated the role of this subset of T lymphocytes in the pathophysiology of sepsis to explain how this disease arises and to inform the development of new therapeutic strategies.[3,8-10]

With respect to these preliminary considerations, the aim of the present article is to present a succinct review of the role of Treg cells in sepsis, focusing on its pathophysiologic features, and to identify perspectives for scientific research into the pathophysiology of this disease via computational modeling/*in silico* experimentation.

METHODS

The present article is a result of a specific search strategy of the following databases: LILACS (Literatura Latino-americana e do Caribe em Ciências de Saúde/*Latin American and Caribbean Health Sciences*), PubMed (National Library of Medicine), and SciELO (Scientific Electronic Library Online). The search terms followed DeCS (Descritores de Ciências da Saúde/*Health Sciences Descriptors*), particularly T lymphocyte subsets and sepsis. The term regulatory T cells was also used, although it is not listed in DeCs. The following strategies were utilized: strategy 1 - T lymphocyte subsets + sepsis; and strategy 2 - Regulatory T cells + sepsis.

The literature search resulted in 1134 citations, as described in table 1, out of which 50 articles were selected. The criteria for selection prioritized the focus on the regulatory role of Treg cells in the immune system and their participation in the pathophysiology of sepsis.

In addition to the selected articles, immunology and internal medicine textbooks were also used for the literature survey as well as important references previously known by the authors.

Table 1 - Number of articles identified in literature searches

Search strategy	Consulted database		
	PubMed	SciELO	LILACS
Strategy1: T-lymphocytes subsets + sepse	918	0	1
Strategy 2: Regulatory T cell + sepse	215	0	0

RESULTS

The data collected from the selected sources were organized under the following headings: "CD4+CD25+ regulatory lymphocytes" and "CD4+CD25+ T lymphocytes and pathogenesis of sepsis". A final short section titled "Computational Frontiers: *in silico* experiments with Treg lymphocytes in sepsis" was also included to present research perspectives in the field of computational modeling, including experiments developed by the authors.

CD4+CD25+ regulatory T lymphocytes

The human organism has countless mechanisms to promote homeostasis, including the immune system, which comprises a complex combination of interacting elements to maintain the balance between immunity and immune tolerance[11], still being responsible for producing an effective response against infectious and non-infectious challenges (e.g., tumors) without triggering autoimmune events or processes that are harmful to the host.[11] In this regard, immune tolerance, i.e., non-responsiveness to a previously recognized antigen, is crucial and occurs with a significant participation by Treg cells.[12]

Origin and suppressive function

Treg cells were initially identified in the early 1970s in murine models and were later discovered in humans. Treg cells are a T lymphocyte subset characterized by the expression of CD4 and CD25 as well as the transcription factor FoxP3 (forkhead box P3) and are indispensable for the control of the immune response to self and non-self antigens by suppressing effector T cells.[13] Sakaguchi et al.[14] observed that adoptive transfer of a T lymphocyte population depleted of CD25+ surface cells induced autoimmunity in several organs and systems, particularly in immunodeficient individuals.[14] The increasing interest in the study of Treg cells in recent years concerns their role in the maintenance of the mechanisms that ensure self-tolerance and the regulation of the immune response. When the T cell subsets that suppress the immune response were initially described, they were designated suppressor T cells because they were believed to be CD8+ T lymphocytes. However, recent studies have shown that suppressor cells participate in the regulation of the immune response but resemble CD4+ rather than CD8+ T cells. The population of CD4+CD25+ T cells includes the Treg lymphocytes, which may minimize

the proliferation of other T cell populations as it has been shown in vitro.[15] The suppressive effects of these cells concern both the adaptive (T and B cells) and the innate (monocytes, macrophages, and dendritic cells) immune response. After activation of the T cell receptor (TCR), natural Treg cells hinder the *in vivo* and *in vitro* immune response in a non-specific antigen manner, by means of a mechanism independent of antigen-presenting cells (APCs) and related to non-restricted major histocompatibility complex (MHC).[6]

Naïve CD4+CD25+ T cells are produced in the thymus in Hassall's corpuscles and are activated upon reaching the peripheral blood and secondary lymphoid organs, thereby acquiring the memory phenotype.[16] These cells represent approximately 5-15% of the CD4+ T lymphocytes in peripheral circulation.[17] The interleukin 7 (IL7) receptor (CD127), which is negatively regulated by the FoxP3 nuclear factor, is currently considered to be the most specific marker for identifying Treg among T lymphocytes and allows the identification of those with greater suppressive capacity.[18]

The mechanisms employed by Treg cells to perform their suppressive function are quite complex and are still the subject of *in vitro* studies. At least three such mechanisms are postulated:[19]

- physical contact (in time and space) between Treg and CD4+ effector cells whereby CTLA-4 (*cytotoxic T lymphocyte antigen 4*) releases inhibitory signals after binding to the CD80 receptor of dendritic cells or activated T cells;[20]

- participation of inhibitory cytokines, such as IL-10 and tumor growth factor (TGF) β1, which have been observed in an *in vivo* study;[21] IL-10 inhibits the activation of APCs, is an antagonist of interferon (INF)-γ, and controls inflammatory processes;[21]

- competition with target cells for growth factors, particularly IL-2, possibly resulting in apoptosis triggered by cytokine deprivation.[19]

In addition to the mechanisms listed above, a fourth possible immunosuppressive mechanism has been described whereby regulatory T cells acquire cytotoxic activity and release granzymes and perforins, leading to the cytolysis of target cells.[22]

It is important to note that effector T cells resistant to suppression by Treg cells have been described.[23]

Groups of Treg lymphocytes

Currently, Treg cells are divided into two groups: natural and adaptive cells.[24]

Natural Treg cells constitutively express surface CD25+ and are therefore designated CD4+CD25+ T lymphocytes.[25] In addition to the CD25 marker, natural Treg lymphocytes express other surface components that, although non-specific, also contribute to the identification of these cells. Some of the most important such components are: CTLA-4, GITR (Glucocorticoid-induced tumor necrosis factor receptor), TNFR-2 (tumor necrosis factor receptor 2), and HLA-DR (human leukocyte antigen).[19] Natural Treg cells are further characterized as CD4+ T lymphocytes that express the alpha chain of the high-affinity IL-2 receptor (CD25) but do not express other markers typical of activated T cells. Indeed, genetic deficiency of the IL-2 receptor or IL-2 itself results in the development of autoimmune diseases.[12] Binding and the resulting paucity of IL-2 is one likely mechanism of suppression employed by Treg cells, as mentioned above. Significant evidence has been found *in vitro* that the effector function of CD4+ T cells is minimized by this mechanism; however, *in vivo* experimental evidence is lacking. This same study also demonstrated *in vivo* that the homeostasis of both Treg and CD8+ T lymphocytes is susceptible to regulation by IL-2.[26]

Although the stimuli that trigger the production and development of natural Treg cells are not fully elucidated, it has been suggested that recognition of self antigens in the thymus mediated by high-affinity TCRs is the signal involved in this process.[12]

Some surface receptors of Treg cells, such as CD27, Fas, CD26L, and the chemokine receptors CCR6, CCR7, CCR8, and CD103, allow for their migration to the sites where inflammation occurs. Nevertheless, these markers reflect the activated state of T lymphocytes and are not specific to the Treg cell subset.[24]

Adaptive Treg cells are produced at peripheral sites under the influence of a myriad of antigenic stimuli or under tolerogenic conditions and exert their suppressive function through the release of cytokines, such as IL-10 and TGF-β.[27] These cells include TR1 (type 1 regulatory cells), TR3, CD4+CD8+ T cells, natural killer cells (NK), suppressor CD8+ lymphocytes, and gamma-delta T cells.[28] TR1 cells can control memory T cells both *in vitro* and *in vivo* and suppress Th1- and Th2-mediated (T helper cells) immune responses to microorganisms, allergens, and oncogenic processes,[29] particularly through the production of IL-10.[30] In turn, the suppressive effects of Th3 cells are non-antigen-specific and mediated through the release of TGF-β,

a widely expressed factor that regulates the functional activity of several immune cell types. Therefore, TGF-β-producing Th3 cells most likely play an important role in several features of immune control and T cell homeostasis.[31]

Role of factor FoxP3

Treg cells may be produced in the peripheral blood by the induction of naïve CD4+ T lymphocytes, which are considered an important source of these cells. Different soluble substances, such as cytokines, retinoic acid, and neuropeptides, lead to increased FoxP3 expression, which facilitates the peripheral generation of Treg cells.[32,33] Hori et al. (2003)[34] showed that the transcription factor FoxP3 is primarily expressed by Treg cells and that naïve T cells transfected with FOXP3 mRNA manifest the properties of regulatory cells.[34] This study also found that the transfected cells assumed a phenotype similar to that of Treg cells, producing cytokines and other molecules characteristic of Treg cells, such as CD25, CTLA-4, CD103, and GIRT, in addition to suppressing the proliferation of other T cells and inhibiting the development of autoimmune disease *in vitro*.[34]

In humans, the FOXP3 gene is located on the short arm of the X chromosome and is primarily expressed by thymus, spleen, and lymph node cells, particularly CD4+CD25+ T cells. The FOXP3 gene synthesizes a transcription factor - the FoxP3 - that increases or inhibits the transcription of specific genes.[24]

FOXP3 deficiency causes severe systemic autoimmune disorders. In addition, effects on the genesis and/or function of FOXP3-dependent Treg cells are associated with the development of rheumatoid arthritis in particular as well as collagenosis and vasculitis, mixed connective tissue disease, Kawasaki disease, Wegener's granulomatosis, systemic lupus erythematous, and Sjögren's syndrome.[35] In humans, mutations of this gene are associated with IPEX syndrome (Immunodysregulation polyendocrinopathy enteropathy X-linked syndrome), which is characterized by enteropathy, diabetes mellitus with destruction of pancreatic islet cells, thyroiditis, and eczema, with lethal outcomes occurring by the second year of life.[19,24,33] Animal studies have shown that some types of CD4+CD25+ T cells hinder the development of autoimmune diseases, such as experimentally induced inflammatory bowel disease, experimental allergic encephalitis, and autoimmune diabetes mellitus. This suppressive mechanism is activated via the TCR; therefore, it is antigen-specific and, consequently, involves contact between the suppressor cell and its target. The clinical implications arising from the suppression of the immune response by Treg cells are remarkable. For example, immunization following the reduction or limitation of Treg cells may increase the immune response to conventional vaccines. Allergic and autoimmune diseases and diseases involving tissue rejection and organ suppression may be treated by increasing the suppressive function of Treg cell subsets.[15,35]

With respect to mechanisms of FoxP3 nuclear signaling in Treg cells, several studies have shown that after antigens bind to the TCR, intracellular signals are attenuated due to the interaction between the nuclear factors NF-κB (Nuclear factor kappa-B) and NFAT (Nuclear factor of activated T cells) and FoxP3, resulting in the inhibition of IL-2, IL-4, and INF-γ transcription and increased expression of CD25 and CTLA-4.[36,37]

Current views

After several decades of doubt and questioning, regulatory cells have finally come to occupy a more central place in the current debates in immunology. Natural CD4+CD25+ Treg cells play an important role in the maintenance of tolerance to endogenous antigens and in the regulation of the immune response against external antigens, thereby protecting the host from injury. A better understanding of these cells may enable their use as adjuvant treatments for numerous conditions related to the immune system, such as autoimmune and allergic illnesses,[38] cancer,[39] primary immunodeficiencies,[24] dermatoses,[40] transplant rejection,[17] rheumatic diseases,[35] and infectious diseases,[41] particularly sepsis. This last topic is discussed next.

CD4+CD25+ T lymphocytes and the pathogenesis of sepsis

The pathogenesis of sepsis is associated with the generalized dysfunction of the immune/inflammatory response, especially cellular immunity, which results in increased morbidity among the affected patients.[42,43] Concomitant with the intense systemic inflammatory response made to reestablish homeostasis, regulatory mechanisms are required to control the inflammatory process. However, evidence suggests that the regulatory mechanisms do not appropriately restrict the

development of the condition, resulting in dysfunction of the innate and adaptive responses during the progression of the disease.[9] The main features corresponding to the pathophysiological mechanisms currently characterized include the following: lymphopenia associated with apoptosis of B and T lymphocytes and dendritic cells;[44] increased numbers of Treg cells at peripheral sites and increased suppressive activity;[8,45] and alteration of the phenotype and function of monocytes, evidenced by the expression decrease of the HLA-DR receptor, the granulocyte-macrophage colony-stimulating factor (GM-CSF), and pro-inflammatory cytokines.[46]

Increased serum levels of anti-inflammatory cytokines such as IL-10 reduce antigen presentation related to the decreased expression of MHCII molecules such as HLA-DR on the cell surface, in addition to apoptosis.[47]

Toll-like receptors (TLR), which recognize pathogen-associated molecular patterns (PAMPs), play an important role in the innate and acquired immune responses. Evidence indicates that TLR are also expressed by Treg cells and orchestrate specific molecular mechanisms that contribute to sepsis.[48] A controlled study conducted with septic patients found increased levels of TLR-2, primarily during infections with *Gram*-positive bacteria, suggesting that TLR tolerance may affect the expression of Toll-like receptors of *Gram*-negative bacteria involved in sepsis.[49] Several other studies have shown that TLR-2 controls the synthesis and release of various cytokines during infection and thereby contributes to the immunopathogenesis of sepsis through complex mechanisms.[50-52] In addition, some alterations in FoxP3 expression caused by several TLR ligands are implicated in the complex immune mechanisms present in sepsis.[53]

Sepsis has been related to flaws in the immune response. Indeed, defects in phagocytosis and the increased production of immunosuppressive cytokines such as IL-10 and CD25+FoxP3+ T cells have been described.[54] One of the first mechanisms causing such alterations is the apoptosis of lymphoid and myeloid cells. A recent study elucidated one further mechanism contributing to this response in infections associated with stimulation by superantigens. Under these conditions, a greater number of effector cells express a regulatory phenotype, and T lymphocytes begin to exhibit a superantigen effect that is dependent on the quantity of cytokines produced.[55] An *in vivo* study that sought to demonstrate the regulation of CD8+ T cell differentiation and expansion by Treg cells mediated by the availability of IL-2 also described the effects of Treg on CD4+ cells. Treg cells were found to limit both the priming of these cells in the lymph nodes and their effector activity at sites of inflammation and to play an important role in cell homeostasis, priming, and memory formation in CD8+ cells.[26] Such immune alterations may contribute to fatal sepsis, particularly as a function of the state of lymphocytic anergy within its pathophysiology.[56] Lymphocytic anergy has also been associated with the development of late secondary infections.[57]

Some studies have shown that CD4+CD25+ T lymphocytes can suppress the adaptive immune response involved in immune dysfunction in sepsis.[9,58]

One study of induced sepsis in rats found a significant increase in CD4+CD25+ T cells in the peripheral blood and the spleen, and the underlying molecular mechanism was associated with the expression of FoxP3 protein which amplified Treg cells in the septic animals.[59] IL-6 was the mediator implicated in the proliferation of CD4+CD25+ T cells, whereas the levels of IL-10 did not vary.[59] Similar findings were described in a study in humans. The percentage of CD4+CD25+ T cells increased significantly in septic patients compared with healthy individuals. This observation was associated with lower expression of the FOXP3 gene and the consequent impairment of lymphocyte proliferation.[9]

Animal models have provided evidence that an insufficient number of Treg cells may contribute to autoimmunity because adoptive transfer of these cells results in positive outcomes. Similarly, under favorable conditions, the production of Treg cells may be stimulated at peripheral sites and thereby protects against the development of autoimmune diseases. In humans, the effect of insufficient numbers of Treg cells is more evident in patients with IPEX syndrome, in whom Treg cells are completely lacking.

In patients diagnosed with these conditions, the main challenge is to measure the number of Treg cells and to establish whether their numbers are insufficient at the site of inflammation. Counting Treg cells is very difficult due to several factors: (1) the definition of which cells should be counted because there is not a known unique marker for Treg cells and (2) the presence of a wide diversity of Treg cell subsets. Although the definition of Treg lymphocytes may be established by analyzing the expression of FOXP3 by flow cytometry, effector T cells have also been shown to express FoxP3 factor, resulting in an impasse because

any quantification of Treg cells by this method may also include recently activated effector T cells.[23]

Because Treg cells are usually implicated in autoimmune diseases, in a recent study, Prots et al.[33] demonstrated that reconstitution of these cells may ameliorate autoimmunity, inflammation, and graft rejection as observed in numerous animal models. These findings represent an encouraging therapeutic perspective and point to the need for achieving a better understanding of the genesis, growth, and function of these cell subsets.[60,61]

COMPUTATIONAL FRONTIERS: *IN SILICO* EXPERIMENTS WITH TREG LYMPHOCYTES IN SEPSIS

Computational simulation, or *in silico* modeling, of the immune system is a recently developed tool that is included in the range of methods available to researchers in immunology. Modeling of the immune system using computational devices serves to better characterize this system and to apply the acquired knowledge in other scientific fields, such as computing and engineering, to the solution of complex problems. Among the features that make the development of *in silico* models relevant, Li et al.[62] observed that there are many medical hypotheses on how the immune system reacts to infections, which must be tested. Consequently, *in silico* models may aid researchers in the understanding of the mechanisms involved in the immune response. In addition, the knowledge gained may be applied to the development of novel treatments, and their efficacy may be tested using the same model. Finally, these authors[62] also state that in addition to being less expensive, *in silico* models take less time to complete than *in vivo* studies.[62]

Among the various approaches to immune system modeling, the system based on autonomous agents, also known as multi-agent systems (MAS), is promising. Some of the advantages of the use of agent-based models include the exploration of the "emergence of complex and deterministic macroscopic functions from stochastic microscopic interactions",[62] i.e., exploration in terms of complexity and the chance/necessity debate.[63] For this reason, it is possible to verify hypotheses on how cells interact and how behaviors emerge from such interactions.

BIS, also known as "The Basic Immune Simulator",[64] and AutoSimune[65,66] are some of the known agent-based immune system simulators. BIS is an agent-based model designed to investigate the interactions between innate and adaptive immune cells. AutoSimune is an extension of BIS designed to test hypotheses of autoimmune diseases.[67]

Based on the above succinct conjectures, simulation of Treg cell behavior might represent an important tool for testing hypotheses and demonstrating their role in sepsis. In this regard, the simulator developed by Possi et al.[65,66] is a natural candidate to perform such simulations. For this purpose, an agent simulating the Treg cell, together with its regulatory behavior on effector T cells, must be included in the model. Moreover, the behavior of the cytokines involved, especially anti-inflammatory cytokines such as IL-10, must be simulated. Another important element to be modeled is the interaction between mast cells and Treg cells since, according to Lu et al.,[68] mast cells, which are already simulated in AutoSimune[69], play a critical role in Treg lymphocyte-dependent peripheral tolerance.[68] We strongly believe that such in silico studies have a great potential to help elucidating the pathophysiology of sepsis.[70]

CLOSING REMARKS

The existence of regulatory T cells that specifically modulate the immune response bears significant clinical implications. There is great interest in demonstrating how the activity of populations of CD4+CD25+ T cells may be increased to minimize undesirable immune responses and how it may be reduced to promote desirable responses.

Because increased numbers of CD4+CD25+ T cells circulating in the peripheral blood of septic patients are associated with a reduced proliferative response, Treg cell counts may represent a simple and valuable biological marker of lymphocytic anergy, which requires further elucidation via *in vivo, in vitro,* and *in silico* experiments. Based on these considerations, the development of new markers to more easily identify Treg cells is of paramount importance because they may contribute to the diagnosis of patients with suspected sepsis.

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RESUMO

A resposta inflamatória sistêmica representa o evento patogênico central da sepse, subjazendo às manifestações clínicas e aos achados laboratoriais presentes nos enfermos. Inúmeras pesquisas têm demonstrado que os linfócitos T CD4+CD25+ - também conhecidos como células T reguladoras (Treg) - participam dos processos de desenvolvimento da sepse, em virtude de sua capacidade de suprimir a resposta imune. Com base nessas ideias, propôs-se, no presente artigo, a discussão do papel dos linfócitos Treg na sepse, com base na revisão da literatura com estratégia de busca definida (LILACS, PubMed e SciELO), tendo em vista duas abordagens principais: a participação dessas células nos processos de inflamação e imunidade, e as perspectivas de investigação fisiopatológica computacional da condição mórbida.

Descritores: Linfócitos T reguladores; Sepse/fisiopatologia; Sepse/terapia; Inflamação; Imunidade; Simulação por computador.

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