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Trichomoniasis immunity and the involvement of the purinergic signaling

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ABSTRACT

Innate and adaptive immunity play a significant role in trichomoniasis, the most common non-viral sexually transmitted disease worldwide. In the urogenital tract, innate immunity is accomplished by a defense physical barrier constituted by epithelial cells, mucus, and acidic pH. During infection, immune cells, antimicrobial peptides, cytokines, chemokines, and adaptive immunity evolve in the reproductive tract, and a proinflammatory response is generated to eliminate the invading extracellular pathogen *Trichomonas vaginalis*. However, the parasite has developed complex evolutionary mechanisms to evade the host immune response through cysteine proteases, phenotypic variation, and molecular mimicry. The purinergic system constitutes a signaling cellular net where nucleotides and nucleosides, enzymes, purinoceptors and transporters are involved in almost all cells and tissues signaling pathways, especially in central and autonomic nervous systems, endocrine, respiratory, cardiac, reproductive, and immune systems, during physiological as well as pathological processes. The involvement of the purinergic system in *T. vaginalis* biology and infection has been demonstrated and this review highlights the participation of this signaling pathway in the parasite immune evasion strategies.

Human trichomoniasis is the most common non-viral sexually transmitted infection caused by the parasite *Trichomonas vaginalis* with an incidence of 276 million new cases each year [1]. The vaginal squamous epithelium is the primary site of infection, although the parasite may also reach urethra and endocervix [2,3]. In men, the parasite leads to urethra infection and the presence of trophozoites in the prostate gland has been demonstrated [2,4]. Updated data revealed that approximately 80% of *T. vaginalis* infections are asymptomatic in both men and women [5–7].

In symptomatic women, the clinical manifestations are vaginal discharge, pruritus, odor, and irritation [3]. The typical vaginal discharge is caused due to intense leukocytic infiltration within the genital tract mainly promoted as consequence of epithelial cell death mediated by inflammation and recruitment of polymorphonuclear leukocytes [8]. In contrast

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to women, the infection in men is in general self-limited, which seems to be associated to the characteristics of male genital fluid enriched in zinc that displays a critical cytotoxic effect [9]. If present, men urethritis is associated with discharge, dysuria, and mild pruritus or burning sensation immediately after sexual intercourse [10].

Studies support that the impact of trichomoniasis is not restrict to vaginal or urethral site infection but also presents a major influence in HIV transmission and acquisition [11,12], in the risk of cervical [13] and prostate cancer [14–16] and is also related to adverse pregnancy outcomes [17,18] and female and male infertility [9,19]. Despite all serious consequences attributed to the *T. vaginalis* infection the treatment is still restrict to a single therapeutic option, the nitroimidazole drug family, mainly represented by metronidazole and tinidazole. Besides the problematic associated to side effects, lack of treatment of sexual partners, inaccurate diagnosis, the increasing number of drug resistance in clinical isolates is relevant [10,20,21]. It has become clear that the impact of trichomoniasis on public health demands early and correct diagnosis, prompt treatment and constant studies on pathogenic mechanisms and host immune response involved in the infection.

The purinergic system constitutes a signaling cellular net that employs purines and pyrimidines as effectors compounds, which may be inactivated by enzymes named ectonucleotidases or uptake by cells through transporters or they can bind to purinoceptors [22]. The purinergic system is involved in almost all cells and tissues signaling pathways, especially in central and autonomic nervous systems, endocrine, respiratory, cardiac, reproductive and immune systems, during physiological as well as pathological processes [23].

In this context, the aim of this review was to highlight the immunological aspects involved in *T. vaginalis* infection, with focus on the purinergic signaling involvement in the parasite immune response evasion mechanisms.

Innate immunity

The female reproductive tract is a particular immunological site that plays a pivotal role on mucosa protection from a variety of pathogens. Besides this crucial function, the mucosal immune system of the female genital tract is in constant adaptation in order to respond to the many physiological processes that take place at this site. Hormone modulation, conception, pregnancy and protection against pathogens are some events that constantly modulate innate and adaptive responses at mucosal level [24]. In comparison with female tract, less information is available regarding the immune system in male urogenital tract, especially due to limitation on sample availability, as impermeable stratified squamous epithelium of the penile urethra difficult the collection [25]. In this sense, it is increasing the number of immunological studies in epithelial cell lines or alternative samples aiming to characterize innate and adaptive immune responses in male reproductive tract.

A distinguishing aspect of the male and female genital tract is the both immune responses activated at these sites: systemic immunity and mucosal immune reaction [24]. The immune responses in the reproductive tract are mediated by interactions between cells, anatomic components and molecules that constitutes the complex microenvironment regulated by sex hormones and specific microbiome [26,27]. Regardless of the site, the development of a multiplicity of immunological mechanisms allowed the host to prevent establishment or dissemination of pathogenic infections.

Barrier protection: epithelial cells, mucus, pH

The epithelial cells and mucus present in the female reproductive tract provide a strong physical barrier that prevent the transmission of microorganisms and specially, sexually transmitted infections [28]. The luminal portion of female reproductive tract is composed of columnar epithelial cells closely connected with tight junctions whose integrity is maintained by many factors as hormones and chemokines [26,29]. In the male reproductive tract, the urethral mucosa and the testes are responsible for immune responses in consequence of cytokines autocrine and paracrine release [30].

Integrating the physical barrier that prevents the pathogen's entry into the female and male reproductive tract, the mucus layer comprises a dense gel phase capable of trapping invasive microorganisms. Mucin family consists of glycosylated proteins that are present and expressed specifically at the apical surface of epithelial cells through genital tract [31,32]. Beyond those features, cervico-vaginal mucus may prevent the transmission of many pathogens because of the low pH, maintained by lactic acid produced by commensal bacteria, mainly *Lactobacillus* spp., in normal reproductive cycle of healthy women [33]. It was demonstrated that an increase in the vaginal pH and anaerobic bacteria are used for the clinical diagnosis of infections including bacterial vaginosis caused by *Gardnerella vaginalis* (pH > 4.5) and the parasite *T. vaginalis* (pH 5.0–6.0) [34]. The cellular and physiological constitution of female reproductive tract, including these complex components dynamically controls the initial process of establishing pathogen infection.

T. vaginalis infection: overcoming the barriers from epithelial cells and strategies of immune response evasion

As an extracellular pathogen, *T. vaginalis* infects the epithelial layer of human reproductive tract and for the success of the colonization and survival of the trophozoites the parasite must adhere to epithelial cells. The host environment is constantly changing because of diverse biological processes and the parasite needs to evade a series of non-specific host defenses, including mucus, the structure of epithelial layer, pH at the genital site, presence of chemokines and another soluble factors.

T. vaginalis is able to traverse the mucus layer first by mucin binding followed by its proteolytic degradation [35]. It was demonstrated that the parasite binds to mucin possibly by a lectin-like adhesion and proteinase action is activated [35]. The continued release of these proteins may contribute to desquamation of epithelial cells, leading to the destruction of monolayers and allowing the penetrance of the parasite [36].

Besides the crucial role on mucosa invasion, cysteine proteases (CPs) are involved in immune evading mechanisms. It has been shown that CPs can degrade all subclasses of host immunoglobulins produced in response to the infection, as well as extracellular matrix proteins and hemoglobin [37–40].

Another interesting advantage for parasitism is the deficient complement system observed in cervical mucus and vagina, as the majority of complement available is maintained by red blood cells only during menstruation [41,42]. Alternative complement activation is a nonspecific defense mechanism against microorganisms, and it has been demonstrated that *T. vaginalis* activates this pathway. At the same time, the parasite avoids the complement lysis as a strategic mechanism to evade immune responses [43]. Iron, an essential nutritional and metabolic element for *T. vaginalis* parasitism presents high levels during menses and seems to be involved in the resistance to complement lysis. High iron concentrations are involved in the upregulation of parasite CPs leading to degradation of complement C3 portion. This particular modulation by iron contributes to the evasion of the immune response [41].

Recognition of pathogens and antimicrobial peptides

Besides the physical protection, the female and male genital immune system evolved the ability to discriminate their own cells and molecular compounds and in consequence the recognition of pathogens. In this context, innate immune recognition is mediated through the expression of the pattern recognition receptors (PRRs), mainly presented by Toll-like (TLRs) and NOD-like (NLRs) receptors. These receptors are involved in prevention and control of invasive pathogens, through recognition of unfamiliar structures to the host [44]. Once stimulated, PRRs mediate direct killing of pathogens, secretion of cytokines, chemokines and antimicrobial peptides and signal for activation of adaptive responses [45,46]. Human female reproductive tract expresses TLRs in vaginal, endocervical, and epithelial cells [47–49]. It was demonstrated that a variety of immune signals are stimulated by the TLRs in the female tract such as release of cytokines IL-6 and IL-8 [48] in endocervical epithelial cells, recognition of invading pathogens [50], immunity in female reproductive tract, and the modulation of intracellular signaling of nuclear factor-κB (NF-κB) [50]. The role of pattern recognition receptors along the male genital tract was recently evidenced [51]. TLR expression is relatively rare in the deeper tissues of the male genital tract except in the penile urethra where a large number of cells express diverse TLRs. These tissues express TLR9, a receptor that detects viral nucleic acids and plays an important role in antiviral immune defense [52].

Antimicrobial peptides (AMPs) are widely expressed by numerous cell types in mucosal epithelia including DCs, macrophages, neutrophils, NK and epithelial cells, and their cell surface expression can be upregulated during initial stages of infection [53]. AMPs have been studied in mucosal secretions and they include small antimicrobial proteins (defensins and cathelicidins) and large proteins (lysozyme, azurocidin, cathepsin G, phospholipase A2, serine leukocyte protease inhibitor, and lactoferrin). Human alpha and beta-defensins are the most abundant AMPs described for the female reproductive tract and are particularly regulated in the upper and lower portions of the tract depending on the menstrual cycle [24]. It was already demonstrated that defensins have anti-microbial properties against different pathogens, including the HIV virus [24]. In male reproductive tract it was already demonstrated the expression of defensins in urethral secretions during *Chlamydia trachomatis* and *Neisseria gonorrhea* urethritis in men [54].

Escaping from pathogen recognition and the regulation of antimicrobial peptides secretion during trichomoniasis

Molecular mimicry is defined as structural, functional or immunological similarities shared between macromolecules found on pathogens and host tissues. The parasite *T. vaginalis* can also coat itself with host plasma proteins to avoid being recognized as strange by the host immune system [2]. This parasite adaptation prevents the recognition of the pathogen-associated molecular patterns (PAMPs) and consequently the immune response triggering such as antigen presentation and complement-mediated lysis will not occur.

Like many other protozoan parasites, *T. vaginalis* displays phenotypic variation as a mechanism of immune evasion. It was already shown the involvement of two surface immunogens (P230 and P270) in the parasite phenotypic variation. The phenotypic variation for P230 is present on the surface of parasites but undergoes conformational changes that prevent the accessibility of the epitope to antibody binding, allowing it to evade antibody response [55]. On the other hand, the surface expression of P270 is based on the presence of dsRNA virus in the trophozoite cytoplasm and iron concentration [56].

Secretory leukocyte protease inhibitor (SLPI) is a kind of antimicrobial peptide and plays an important role in male and female mucosal protection. *T. vaginalis* cysteine proteases are able to degrade SLPI and turning it non-functional [57]. It was already demonstrated that SLPI levels in the female genital tract are reduced in a *T. vaginalis*-dependent manner [58]. SLPI participates in the prevention of HIV transmission through the inhibition of virus entry into monocytic cells *in vitro* [59]. These data together partially explain the observed increase in risk of HIV acquisition in women with trichomoniasis [11,12].

Zinc is a known antimicrobial defense of the male lower urinary tract against infections. The oxidative nature of the male genital tract combined with high zinc concentration (2.3–15.3 mM) in human prostatic secretions may be an important defense against *T. vaginalis* infection. It is hypothesized that zinc is inhibitory to certain pathogenic factors in the parasite and is also toxic to the trophozoite [60]. An interesting investigation demonstrated that there are variations in zinc sensitivity between different infecting *T. vaginalis* isolates or in the zinc content of host prostatic secretions [60] which may impact symptomatology, spontaneous cure rates, and chronification of the infection in men [60]. More recently, a proteome study revealed that variations in the zinc concentrations are able to induce differential expression of the *T. vaginalis* proteome corroborating that this environmental molecule plays a pivotal role in parasite survival in the adverse environment of male urinary tract [61].

Still concerning antimicrobial peptides, it is well established that superoxide radicals, nitric oxide (NO) and other reactive nitrogen species released by immune effector cells are essential cytotoxic mediators against a diversity of microorganisms including parasites. It was already demonstrated that NO products released by macrophages are cytotoxic to *T. vaginalis* suggesting an important role to these immune cells in the host defense mechanism against the parasite [62]. In addition, the stimulation of macrophages with live *T. vaginalis* induces an increase on NO production and expression of inducible NO synthase (iNOS) levels [63]. Reactive nitrogen radicals may also have a role in limiting *T. vaginalis* infection, as the levels of these mediators and iNOS protein are different in leukocytes and vaginal washes of healthy, symptomatic or asymptomatic women [64]. Neutrophils are the major cells recruited to the infection site, mediating the initial inflammatory response after an acute *T. vaginalis* infection [65] and also mediate the release of NO under parasite stimulation condition [66]. Regarding reactive oxygen intermediates, it was already demonstrated that their production is detected after neutrophils and trichomonads coincubation as the participation of these molecules in these immune cells apoptosis cascade [67].

Innate immune cells in female and male reproductive tract – presence, function and immune response modulation

Neutrophils are present throughout the female reproductive tract with highest numbers in the uterine tubes and gradually decrease from the upper female reproductive tract into the vagina [24]. Neutrophils express TLRs 1–9 and respond to pathogens through phagocytosis, production of oxidative compounds, release of antimicrobial peptides (defensins, phospholipases) and cytokines. They represent an important innate immune defense once the epithelial barrier is disrupted. Epithelial cells of both the upper and lower reproductive tracts produce abundant amounts of IL-8, which is a leukocyte chemoattractant factor [68]. The number of neutrophils varies in the endometrium portion during menstrual cycle but remains stable in the vagina, regardless of the period [68].

Important phagocytic cells in innate immune cells are macrophages and DCs, which represent around 10% of the leukocytes present in the female reproductive tract [68]. Macrophages and DCs are competent antigen presenting cells (APCs) crucial for the induction of adaptive immune responses during infection [69]. Natural killer cells possess cytotoxic activity and constitute about 70% of mucosal leukocytes in the endometrium [68]. Similar to blood NK cells, uterine NK cells produce pro-inflammatory cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-10, IL-8 and IFNγ and thus stimulate the inflammatory response, inducing macrophage activation and cytotoxic T cell generation [28,68,70].

In relation to male reproductive tract, many types of immune cells are present in the urethra and testes, with a prevalence of macrophages, neutrophils, NK and mast cells [25]. In relation to immune functions, mast cells upregulate monocyte chemoattractant protein-1 and thereby recruit macrophages into the testes [25]. Both CD8 and CD4 T cells are present in the epithelium and lamina propria of the urethra with a more abundant presence of CD8 T cell. Based on these studies, it is clear that the lower male reproductive tract is an immunologically competent site that is capable of generating both cellular and humoral immunological responses. However, further studies into their functions in immune responses in the male reproductive site need to be carried out since their role in many immune processes remains unclear.

Cytokines and chemokines are chemical messengers that maintain the normal homeostatic environment and regulate many innate and immune functions in the female and male reproductive tract [28]. The secretion of these molecules produces a hostile environment to pathogen survival either by the prompt communication between the different immune cell types or by the direct antimicrobial nature of these compounds. Several studies have demonstrated the constitutive secretion of numerous cytokines, including GM-CSF, granulocyte colony-stimulating factor (G-CSF), TNF-α, IL-1, IL-6, leukemia inhibitory factor (LIF), TGF-β, and of chemokines such as MIP-1β, monocyte chemoattractant protein-1 (MCP-1), and IL-8 by epithelial cells from the cervix, uterus, and fallopian tubes [48,71].

In the male reproductive tract, the cytokines and other immune regulatory factors are mainly produced in the testes by somatic cells, involved in the regulation of spermatogenesis and other testicular cell functions [25]. Cytokines and other immune regulatory factors are released by various immune cells present in the male genital tract, including macrophages, monocytes, lymphocytes, DCs, and in response to invasive antigen and pathogens. Chronic inflammation and infection conditions also display an impact on the release of these chemical molecules. In both female and male reproductive tracts, cytokine expression and release are regulated by multiple factors, including steroid hormones, the redox system, and prostaglandins [71].

T. vaginalis infection: suppressing cell immunity and the effects of immune mediators during infection

The immunomodulatory responses to *T. vaginalis* infection have been studied *in vitro* with cervical and vaginal epithelial cell lines in association with various immune cell types. As a mucosal pathogen, *T. vaginalis* must adhere to epithelial cell monolayer and once in contact with host cells, the parasite undergoes a drastic morphological shift that leads to tight association to the target cells [72]. The TLRs expression plays a significant role in the innate and adaptive immune responses in epithelial cells, particularly TLR4 [73]. *T. vaginalis* infection stimulates cells through TLR4 pathway, indicating a possible immune mechanism mediated in the epithelial cells during the parasite infection [73].

The outer layer membrane of *T. vaginalis* is covered by an abundant glycoconjugate, the lipophosphoglycan (TvLPG). The TvLPG is a component of the glycocalyx formed by different carbohydrate-associated molecules that binds to galectin-1 and -3 receptors in the host cells [74,75]. TvLPG plays a role in the adherence and cytotoxicity to human cervical cells and modulates inflammatory responses of epithelial cells and macrophages [76]. It was already demonstrated

that TvLPG induces inflammatory response upon contact with human cervical and vaginal epithelial cells by the release of chemokines as IL-8 and macrophage inflammatory protein-3α (MIP-3α). In opposition with TNF-α release it was shown that this mechanism is independent of TLR4 expression [76]. To date, trichomonad ligands for TLR4 have not been identified and host receptors mediating these effects remain elusive. More recently, it was demonstrated that galectin-1 and -3 are expressed by the human cervical and vaginal epithelial cells [75]. The study demonstrated that galectin-1 suppressed IL-8, MIP-3α and RANTES chemokines that facilitate recruitment of phagocytes, which can eliminate extracellular protozoa or bridge innate to adaptive immunity [75]. In addition, the investigation of inflammatory reaction triggered by *T. vaginalis* in human prostate epithelial cell line was already conducted. Co-incubation of prostate cell and parasites increased the expression of the inflammatory mediators IL-1β, CCL2, and CXCL8 [77]. Medium conditioned by incubation of prostate cells with *T. vaginalis* trophozoites contained IL-1β and stimulated the migration of human neutrophils and monocytes [77].

Activation of chemokines and pro-inflammatory cytokines in host immune cells during *T. vaginalis* infection with an important role in innate and adaptive immunity have been widely reported [78–82]. Neutrophils are the predominant inflammatory cells found in the vaginal discharges of patients infected with *T. vaginalis*. It was demonstrated that live trophozoites can induce IL-8 production in neutrophils and that this mechanism may be mediated through the NF-κB and mitogen-activated protein kinases (MAPK) signaling pathways [79]. This finding helps to explain how neutrophils accumulate or mediate initial inflammatory response after acute *T. vaginalis* infection. Neutrophil apoptosis induced by live *T. vaginalis* is mediated by the activation of caspase-3 and reduced expression of the neutrophil anti-apoptotic protein, induced myeloid leukemia cell differentiation protein (Mcl-1) [82].

The recruitment of macrophages to the genitourinary tract is also a critical component for host immune defense against *T. vaginalis*. Trichomonads can stimulate macrophages and DCs leading to production of immunosuppressive cytokines such as IL-10 and TGFβ [83]. Similarly to neutrophils, in macrophages, caspase-3 has been related to the phosphorylation of p38 MAPK signaling cascade which is located downstream of mitochondria-dependent caspase activation [81]. Inflammatory responses induced by *T. vaginalis* in macrophages are also associated with NF-κβ activation dependent on Iκβ-α degradation. Further, the nuclear translocation of NF-κβ was inhibited by the parasite and in turn diminished the production of IL-12 and TNF-α in response to stimulus [83]. Collectively these molecular mechanisms indicate that the effects of *T. vaginalis* on NF-κβ regulation are critical for the production of cytokine and macrophage survival, consequently suggesting an existence of a *T. vaginalis*-induced immune evasion from macrophage attack.

The macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine that modulates innate immunity in inflammatory responses [84]. MIF promotes the production of other proinflammatory mediators, such as TNFα, NO, and prostaglandin E_2 [84]. *T. vaginalis* macrophage migration inhibitory factor (TvMIF) is 47% similar to human macrophage migration inhibitory factor (HuMIF). The presence of anti-TvMIF antibodies was detected in sera from patients infected with *T. vaginalis*, especially in men [85]. Considering that chronic trichomoniasis has been associated with increased risk of prostate cancer [15,86], these relevant data indicate that chronic *T. vaginalis* infections may result in TvMIF-driven inflammation and cell proliferation, thus triggering pathways that contribute to the promotion and progression of aggressive prostate cancer.

The adaptive immune response to trichomoniasis

The human infection by *T. vaginalis* results in specific serum and local antibodies production, although there is little evidence on its efficacy in the parasite *in vivo* clearance [87–89]. The specific IgG against *T. vaginalis* augmented LTB4 production by neutrophils via the crucial complement common pathway activation. Recently, a direct correlation between the reduction of specific serum IgG anti-*T. vaginalis* response detected by ELISA and the effective metronidazole therapy was shown [90]. These findings may contribute to the search for new diagnostic tools and techniques for trichomoniasis in clinical samples.

In the last 40 years the presence of anti-*T. vaginalis* antibodies (IgA, IgM, and IgG, and its subclasses) in serum and cervicovaginal secretions has been demonstrated by radioimmunoassay, ELISA, and immunofluorescence methods [88–95]. IgE, when found in the genital tract, occurs in low levels [89]. Nevertheless, in general is consensus that humoral immunity is not long-lasting, and *T. vaginalis* cysteine proteases secreted in the vaginal secretions and serum of symptomatic women could degrade the levels of IgG, IgM, and IgA [36]. This lack of effective and persistent humoral immunity is also one of the causes of the difficulty in obtaining a vaccine for trichomoniasis [96].

In a detailed study on TvLPG structure, Singh et al. (2009) [97] revealed a dominant role for the CPI-GC core in the induction of chemokine production, NF-κB and extracellular signal-regulated kinase (ERK)1/2 activation in human cervicovaginal epithelial cells. Besides, considering the TvLPG role in trichomoniasis virulence, recently, a study showed IgG antibody response to conserved TvLPG antigen by testing serum and vaginal samples from infected women [95]. In addition, these women with normal pregnancies had higher vaginal IgG anti-TvLPG levels than infected women with adverse pregnancy outcomes. The conserved surface polysaccharide, poly-*N*-acetylglucosamine (PNAG), produced by bacteria, fungi and protozoal parasites was also found in *T. vaginalis* by using antigen-specific human IgG1, indicating an evolutionary convergent acquisition of PNAG synthesis with significance for microbial biology [98].

Involvement of the purinergic signaling in trichomoniasis immune response

Nucleotides can be found in the extracellular spaces and they are mainly released by cells in physiological situations of

active metabolism or stress, anoxia, or injury [99–101]. Extracellular nucleotides and nucleosides can act as signaling molecules through binding to specific receptors, named purinoceptors, identified as P1 and P2 [102]. Enzymes located on the cellular surface named ectonucleotidases are involved in extracellular nucleotide hydrolysis. These enzymes include the E-NTPDase family (ectonucleoside triphosphate diphosphohydrolase), the NPP family (ectonucleotide pyrophosphatase/phosphodiesterase), alkaline phosphatases, and ecto-5′-nucleotidase [103–105].

Purinoceptors and enzymes are expressed in immune cells and the activation of these proteins alters the cellular immune function [23]. ATP can act as a potent proinflammatory molecule that promotes chemotaxis and degranulation of mast cells, neutrophils and eosinophils, besides to activate pain receptors and to increase the release of pro-inflammatory cytokines [23,106–108]. In contrast, the final product of ATP break down, adenosine, presents anti-inflammatory effect, in general because of the predominant expression and activation of A2A receptor in monocytes/macrophages, DCs, neutrophils, endothelial and epithelial cells, eosinophils, lymphocytes, NK cells, and T-natural killers [109].

Among parasites, possible physiological functions are attributed to enzymes involved in the nucleotide degradation, related to protection from cytolytic effects of extracellular ATP [110]. Our research group has investigated the purinergic system in *T. vaginalis* biology mainly through the study of ectonucleotidases. The biochemical characterization of the complete enzyme cascade that breaks down ATP to adenosine by NTPDases and ecto-5′-nucleotidase with degradation of adenosine to inosine by adenosine deaminase (ADA) has been demonstrated [111–113]. Recently, we showed that five putative *TvNTPDases* (*TvNTPDase1–5*) were expressed by both fresh clinical and long-term grown *T. vaginalis* isolates [114].

In addition, the regulation of trichomonads enzymes has been studied. Our findings demonstrate that biochemical NTPDase activity (ATP and ADP hydrolysis) and ecto-5′-nucleotidase are responsive to the serum-restrictive condition and the gene expression of TvNTPDases was mostly increased, mainly *TvNTPDase2* and 4 [114,115]. The same enzymes activation effect could be observed when guanine nucleotides hydrolysis was tested in parasites under serum restriction, with higher adenosine uptake by parasites than guanosine uptake [116]. These results indicate the preference for adenosine by trichomonads, and are in agreement with previous published data [117].

Besides serum restriction, the influence of iron on extracellular nucleotide hydrolysis in *T. vaginalis* isolates from female and male patients was evaluated [118]. Iron from hemoglobin and hemin significantly increased NTP-Dase activity in fresh clinical *T. vaginalis* isolates from female patients and conversely, reduced the enzyme activity in isolates from male patients. Collectively, these results show the influence of iron in trichomonads ectonucleotidases through ATP degradation and adenosine production [118]. The ADA profile in different *T. vaginalis* isolates treated with different iron sources or with limited iron availability was also evaluated. We found a reduction in activity and an augment in ADA gene expression after iron restriction by 2,2-bipyridyl and ferrozine chelators [119]. These data support the hypothesis that iron can modulate the activity of the enzymes involved in purinergic signaling and it is implicated in establishing infection and parasite survival.

Further the enzymes regulation, we were interested to investigate whether the purinergic signaling would be involved in the innate immune response during trichomoniasis. Indeed, the *T. vaginalis* trophozoites were able to induce NO synthesis in neutrophils through iNOS pathway [66]. Importantly, adenosine and inosine promoted reduction on NO secretion by trichomonads stimulated-neutrophils and this immunosuppressive effect of the nucleosides occurred via A2A receptor activation. Moreover, the *T. vaginalis* ecto-5′-nucleotidase activity appears to play a key role in adenosine generation, indicating the efficiency of the purinergic cascade in the process of immune response evasion by the parasite [66].

Considering the increasing metronidazole resistance displayed by *T. vaginalis* isolates, it is imperative to search for new treatment alternatives [21]. Several isolated compounds and natural products have been tested *in vitro* against *T. vaginalis* with some success although no compound demonstrated efficacy better than metronidazole to rouse the pharmaceutical industry interest [120]. We have characterized the cytotoxic effect of the Amaryllidaceae alkaloids, candimine and lycorine, against *T. vaginalis*, as well as the mechanism of cellular death [121,122]. Taking into account that both alkaloids were active against the trophozoites, we tested the effect of compounds on ectonucleotidases. Indeed, NTPDase and ecto-5′-nucleotidase activities were strongly inhibited by candimine and lycorine on 24 h-treated parasites, although the effect was abolished when treated parasites were inoculated in new culture medium without alkaloid [123]. Considering the proinflammatory role of ATP, which levels are accumulated during the alkaloids treatment, the regulation of extracellular nucleotide levels could be relevant in increasing susceptibility of *T. vaginalis* to host immune response in the presence of lycorine and candimine [123]. The adjuvant inflammatory effect produced by antitrichomonal agents could be an interesting approach to the search to alternative treatments for trichomoniasis, especially for the metronidazole resistant cases.

Final considerations

It is undoubted the important impact of trichomoniasis in public health, including the serious consequences derived from this STD and the costs in the healthcare system associated to *T. vaginalis* infection. Unfortunately, trichomoniasis is not a reportable disease and increasing failures in diagnosis and treatment are reported. Efforts have been made to understand how *T. vaginalis* succeeds parasitism and infection, and the studies on the immunological aspects of the disease have brought considerable advances that contribute to the comprehension of host-parasite relation. The main immune response during infection is the innate immunity, and the adaptive response is also elicited although it is not persistent. So far, we showed the involvement of the purinergic signaling in *T. vaginalis* infection in the NO production by neutrophils and the regulation of NTPDases and ADA by iron. Moreover, a perspective on the adjuvant effect of anti-*T. vaginalis*

compounds that modulate immune responses is a potential alternative for trichomoniasis treatment. Studies should be encouraged to foment the knowledge on immunological aspects of this STD with the great aim to achieve the reduction of *T. vaginalis* burden.

Conflicts of interest

All authors have declared that are no conflicts of interest.

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