UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
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TRABALHO DE CONCLUSÃO DE CURSO DE FARMÁCIA

Infinity war: *Trichomonas vaginalis* and interactions with the host immune response

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Infinity war: *Trichomonas vaginalis* and interactions with host immune response

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ABSTRACT

Trichomonas vaginalis is the pathological agent of the human trichomoniasis, with an incidence of 156 million cases worldwide. Due to the increasing resistance of isolates to approved drugs and the clinical complications that include the increase in the acquisition and transmission of HIV, the adverse outcomes during pregnancy, and cervical and prostate cancer, the understanding of the pathogen interaction with the host immune response becomes essential. Production of cytokines and cells of innate immunity: Neutrophils and macrophages are the main cells involved in the fight against the parasite, while IL-8, IL-6 and TNF-α are the most produced cytokines in response to this infection. Clinical complications: *T. vaginalis* increases the acquisition of HIV, stimulates the invasiveness and growth of prostate cells and generates an inflammatory environment that can lead to preterm birth. Vaccine candidate targets: Adhesion proteins, cysteine peptidase, and α-actinin are currently cited as candidate targets for a vaccine development. Antibodies: IgG and IgG1 was found in serum samples of rodents infected with isolates from symptomatic patients as well as patients with symptoms. However, this antibodies production does not protect against a reinfection. Endosymbiosis: *Mycoplasma hominis* increased the cytotoxicity, growth, and survival rate of the parasite. In this context, the understanding of the mechanisms involved in the host-*T. vaginalis* interaction that elicit the immune response can contribute to the development of new targets to combat trichomoniasis.

Introduction

The unicellular protozoan *Trichomonas vaginalis* is the etiologic agent of human trichomoniasis, the most common non-viral sexually transmitted infection (STI) in the world, with higher numbers of incidence than *Chlamydia* infection, gonorrhea, and syphilis. In 2016, the World Health Organization (WHO) estimated an incidence of 156 million cases worldwide [1]. Both men and women can be asymptomatic. In women, when symptoms occur, they are manifested by vaginal discharge, itching, dysuria, and abdominal pain. In addition, *T. vaginalis* infection can affect vaginal pH, which increases from 4.5 to >5, which partially explaining the association between the parasite, bacterial vaginosis (BV), and candidiasis. In men, the most common symptoms are prostatitis, decreased sperm motility, and epididymitis. In addition, several studies have demonstrated that *T. vaginalis* infection can increase the acquisition and transmission of the HIV in men and women. Co-infection with *Chlamydia*, gonorrhea, syphilis, and herpes simplex virus type 1 and 2 have also been described [2,3].

Currently, there are three drugs approved by the Food and Drug Administration (FDA) against trichomoniasis: secnidazole, metronidazole (MTZ) and tinidazole, representatives of the 5-nitroimidazole class. However, the rate of resistant isolates is an increasing problem [3]. The first metronidazole-resistant isolate was detected in 1962, two years after its introduction [4]. Likewise, the newest therapeutic alternative, secnidazole, already has resistant isolates [5]. Furthermore, the test with the highest sensitivity and specificity for the detection of *T. vaginalis* known as nucleic acid amplification tests (NAATs) are not a routine test in Brazil, which, added to the fact that it is not a notifiable STI obligatory, contribute to the underdiagnosis and, consequently, underestimation of trichomoniasis cases [6].

In this context, the understanding of the mechanisms involved in the interaction between *T. vaginalis* and the host immune response can contribute to the development of new targets to combat the parasite. Thus, the search strategy was based on the combination of *Trichomonas vaginalis* or trichomoniasis with different keywords: immune, immunity, macrophages, neutrophils, immune response, innate immunity, adaptive immunity, pregnancy, antibody, *M. hominis*, vaccine and HIV. Only articles indexed in the PubMed Central® database, in english or portuguese and published between 2000 and 2022 were included in this review, in order to summarize the most current data on the trichomoniasis-immune response field.

1. The avengers: the cells of the host immune system and cytokine production

During an infection, host defense begins with innate immunity mediated by myeloid cells, natural killer cells, innate lymphoid cells, the complement system, and defensins [7]. Neutrophils are polymorphonuclear cells (PMNs), one of the most important members of the immune response, which can be recruited by pattern recognition receptors present on endothelial cells or inflammatory mediators released by resident leukocytes in infected tissues [8].

IL-8 is a proinflammatory cytokine which can regulate the activation, migration, and degranulation in neutrophils, being released under inflammatory stimulations such as lipopolysaccharide (LPS), present in *T. vaginalis.* Adhesion or contact between the parasite and neutrophils is essential for the cytokine production, as well as the membrane integrity of the protozoan, since lysates of trichomonads and *T. vaginalis* excretory-secretory products (TvSP) were not able to induce the same IL-8 amount. A likely pathway is through the regulator of cytokine transcription, factor nuclear kappa B (NF-

kB), and mitogen-activated protein kinase (MAPK) [9]. Although secretory products did not stimulate neutrophils in the same way as live trichomonads, they also contribute to the IL-8 production due to the lipid mediator leukotrienes (LT) B4 in their composition. Through high affinity (BLT1) and low affinity (BLT2) LTB4 receptors present on neutrophils, the NF-kB and cAMP response element-binding (CREB) pathway are activated, leading to the cytokine production [10,11].

The pathogen-neutrophil interaction goes beyond the cytokine production. This direct contact decreased the myeloid cell leukemia 1 (Mcl-1) expression, an anti-apoptotic protein, and increased the caspase-3 expression, a protein related to spontaneous apoptosis in neutrophils, triggering the acceleration of the apoptosis process [12]. Interestingly, while live trophozoites enhanced the rate of neutrophil apoptosis, trichomonads lysate reduced it. *T. vaginalis* lysate co-incubation with PMNs maintained Mcl-1 expression for longer time than the control group with a reduction in caspase-3 activation observed at 15 and 25 hours incubation, while caspase-3 cleavage by the untreated group occurred at 15 hour incubation [13]. Caspase-3 can be activated by the intracellular increase of reactive oxygen species (ROS) by neutrophils in response to the presence of trichomonads, via the NADPH oxidase system [14]. Trichomonads lysate, excretory-secretory product, and membrane component stimulated superoxide anion production, in contrast to the reduction of this production by the protozoan peptidase inhibition and release of ROS and IL-8 by blocking adenosine deaminase, the enzyme that converts adenosine into inosine. Meanwhile, adenosine and inosine, anti-inflammatory nucleosides, decreased nitric oxide (NO) generation via iNOS [15-17]. Furthermore, the parasite was able to degraded NO in anaerobic conditions through A-type flavoprotein, an NADH-dependent enzyme which responds to NO species exposure, and overexpressed TvMIF during nutrient stress and serum starvation, inhibiting ROS

production and avoiding its apoptosis [18,19]. Interestingly, reactive nitrogen intermediates (RNI) may be associated with the infection establishment. Isolates of *T. vaginalis* from symptomatic patients inoculated into mice generated higher levels of RNI in vaginal tissue than isolates from asymptomatic ones; in contrast, leukocytes showed higher RNI and iNOS protein band intensity in the asymptomatic group. On the other hand, RNI in vaginal washes and plasma demonstrated to be higher in the asymptomatic group [20,21].

As mentioned earlier, epithelial cells can stimulate neutrophil migration into injured tissues. In this context, it was demonstrated that vaginal epithelial cells (VECs) in the presence of *T. vaginalis* increased IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1) mRNA expression, raising the migration of neutrophils and mast cells. In turn, mast cells stimulated neutrophil migration [22]. The same was observed in ectocervical and endocervical epithelial cells in response to LPS, by producing IL-8 and macrophage inflammatory protein (MIP)-3α, necessary for the dendritic cells maturation [23]. Curiously, when *Prevotella bivia,* bacteria of the vaginal microbiota, was in contact with the protozoan, the same IL-8 production was detected, but MIP-3α induction was reduced, reinforcing the importance of further studies that consider the role of the microbiota in immunodulation during trichomoniasis [24].

VECs and human prostatic epithelial cells in contact with *T. vaginalis* or rTvα-actinin 2 enhanced IL-10, IL-12, IL-6, and Tumor Necrosis Factor alpha (TNF-α) production, while the stimulation of dendritic cells with LPS enhanced CD80, CD86 and major histocompatibility complex (MHC) activation makers, in opposition to rTvα-actinin 2 which diminished MHC-II expression. Likewise, dendritic cells that were stimulated with LPS and later with TvSP decreased the expression of MHC-II. Nevertheless, when TvSP was present, even with previous LPS stimulation, IL-10 production increased and IL-12

reduced. iTregs incubated with mouse bone marrow derived dendritic cells stimulated with LPS and treated with rTvα-actinin 2 showed greater release of IL-10, TGF-β and IFN-γ, suggesting that rTvα-actinin 2 may act as an immunomodulator [25,26].

Macrophages are also members of the innate immune system and play a crucial role in the host defense against pathogens. These cells can secrete cytokines, defensis, ROS and NO [27]. Thus, the influence of macrophages during *T. vaginalis* infection was also explored.

Macrophages in the presence of apoptotic neutrophils induced by *T. vaginalis* raised IL-10 production and decreased IL-6 and TNF-α [28]. However, in the presence of lysate, opsonized or live trichomonads, macrophages enhanced IL-1β, IL-6 and TNF-α levels. This latter is dependent on the activation of NF-kB activation by NO [29]. *T. vaginalis* adhesion in macrophages is associated with cytokine production. In the first minutes of the adhesion process was observed a transient degradation of IkB-α and NF-kB activation, since IkB-α prevents the binding of NF-kB with its sites. However, at a longer adhesion time, inhibition of NF-kB and consequently reducing in the TNF-α and IL-12 production were observed, suggesting a possible pathway that the parasite uses to avoid the generation of cytokine [30]. In mouse macrophages, cytokine production was associated with the toll-like receptor (TLR)-2. The presence of the parasite leads to the phosphorylation of NF-kB p65, p38 and ERK via TLR-2. Activation of the latter resulted in an upregulated expression of the proinflammatory cytokines IL-6, TNF-α and INF-γ, while the NF-kB translocation has been associated with the regulation of NO production and proinflammatory gene expression [31].

During macrophage apoptosis induced by *T. vaginalis*, the activation of caspase-3 led to a downregulation of Bcl-xL, an anti-apoptotic protein, contributing to apoptosis. On the other hand, an overexpression of Bcl-2 did not protect macrophages against

apoptosis, nor impact cytochrome c release or Bax and caspase-3 activation [32]. The release of cytochrome c from mitochondria activated the caspase cascade involving caspase-9 and -3, which in turn, activate p38 MAPK and result in host cell death [33].

Finally, immune system cells also have their ways of preventing the establishment of *T. vaginalis* infection. In mice, trichomonads trigger neutrophils extracellular traps (NET) via ROS generation, ERK1/2 and p38 MAPK pathway. However, cells necrosis was not able to trigger this mechanism [34]. Likewise, human monocytic cells used extracellular traps (ET) to capture *T. vaginalis* through the same pathway previously proposed. Furthermore, in this context, both live and dead parasites were capable of inducing ET formation [35]. Nevertheless, a few years earlier, the death of the parasite was shown to be independent of NETosis. The contact between PMNs and *T. vaginalis* was necessary to kill the parasite via trogocytosis with important participation of serine peptidases and components present in human serum. The serine peptidase within the granules can nibble inside PMN-*T. vaginalis* junction microenvironment, while the serum factors may opsonize the trophozoite, aiding in the death process [36].

Trichomonas vaginalis has been shown to be able to stimulate vaginal, ectocervical and endocervical epithelial cells to release cytokines that can recruit and activate innate immune cells, such as neutrophils, macrophages and mast cells to the site of infection. At the site, the parasite has a complex machinery to modulate ROS, cytokines, and chemokines production as well as to induce apoptosis of the host immune cells, besides interfering in the maturation of antigen-presenting cells, such as dendritic cells. All together, these mechanisms allow the pathogen survival and the infection establishment. Fig.1 summarizes the main mechanisms discussed in this topic.

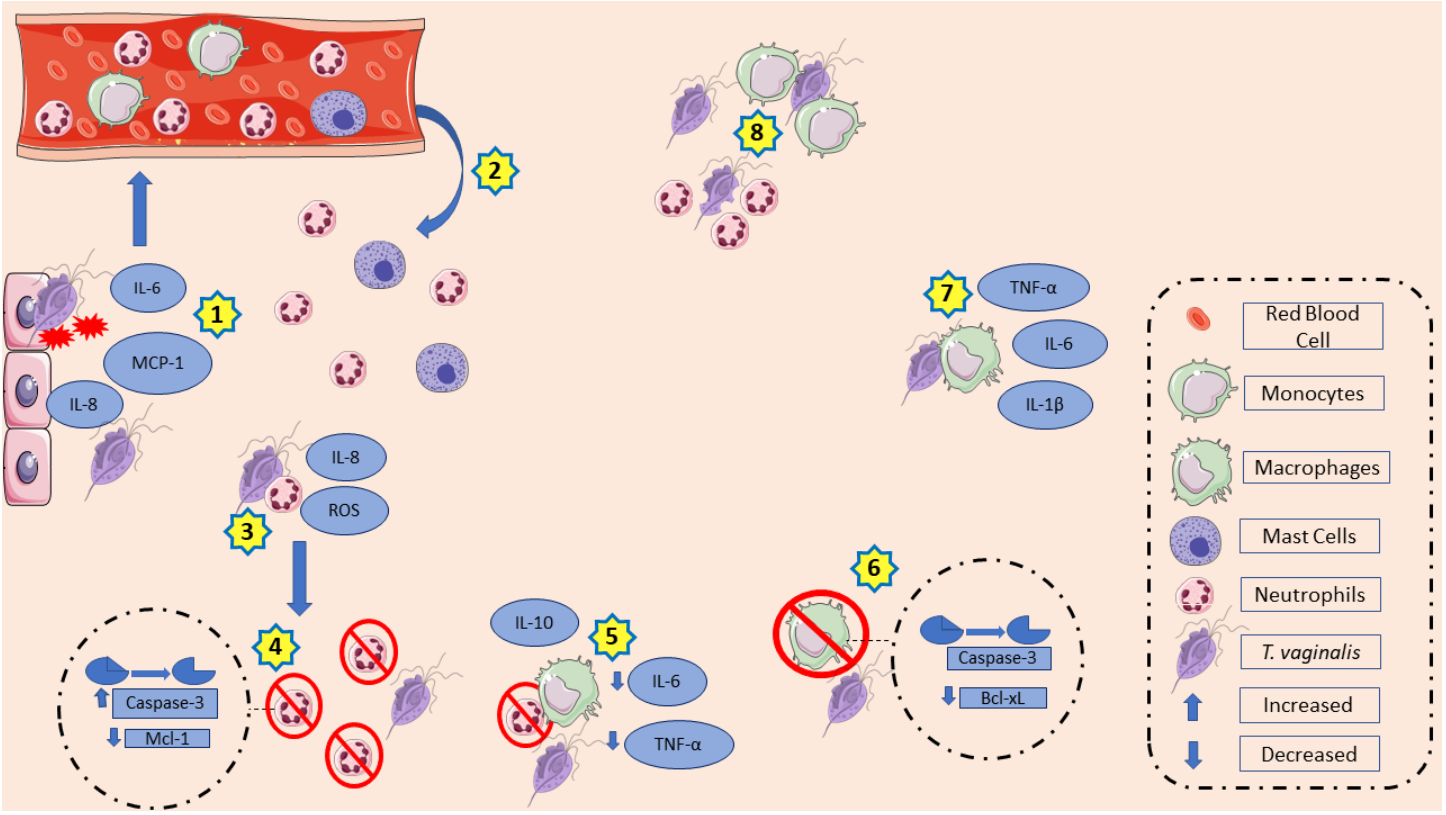

Figure 1. *T. vaginalis* and the interaction with host immune cells. *T. vaginalis* stimulates vaginal, ectocervical and endocervical epithelial cells, releasing IL-6, MCP-1 and IL-8 (1), resulting in the migration of mast cells and neutrophils to the site of infection (2). There, neutrophils in contact with trichomonads release IL-8 and ROS (3) that activate caspase-3 and decrease Mcl-1, an anti-apoptotic protein (4). Macrophages in contact with neutrophils killed by parasite-induced apoptosis, decrease IL-6 and TNF-α and increased IL-10 (5). The interaction between macrophages and *T. vaginalis* can lead to their apoptosis by caspase-3 activation and downregulation of Bcl-xL (6) or result in the release of TNF-α, IL-6, IL-1β (7). Finally, monocytes can kill the protozoan through extracellular traps, while neutrophils use a mechanism known as trogocytosis (8).

2. War consequences: HIV association, outcomes during pregnancy and prostate cancer

Trichomonas vaginalis presence has been associated with several clinical complications such as prostate cancer, preterm birth and increased acquisition and transmission of HIV [37]. Due to the importance of this subject, the next topics will focus on the main immunological mechanism described in the last two decades, exceptionally the paragraph “HIV association” included articles outside this criterion due to relevance.

An infection with *T. vaginalis* increased the chances of acquiring HIV by 1.5 times as compared to uninfected individuals. In addition, HIV-reagent women demonstrated trichomoniasis prevalence of 17,4-20% with high rates of repeat infection [38,39]. Some mechanisms can explain this association. *T. vaginalis* promotes alteration in the vaginal microbiota by reducing lactobacilli producers of H_2O_2 causing an increase in pH and favoring bacterial vaginosis. In turn, BV enhanced pro-inflammatory cytokines that disrupted the mucosal barrier while trichomonads by themselves disrupt the integrity of the cell monolayer generating perforation. Both mechanisms facilitated the HIV-1 passage. The immune response activation due to the *T. vaginalis* infection is responsible for the recruitment of CD4 lymphocytes, the target cell of HIV, as well as peripheral blood mononuclear cells (PBMCs) that released TNF-α, partially involved in enhancing viral replication [40-44].

The protozoan reduced the secretory leukocyte protease inhibitor (SLPI) concentration while its cysteine peptidases cleaved the SLPI, one of the defense mechanisms against the establishment of HIV infection [43,45]. However, a cross-sectional study found no association between the presence of *T. vaginalis* and the enhancement of the expression or activation of CD4+ cells of HLADR+CD38+ phenotypes cells, in disagreement with the aforementioned studies that suggested it as one of the pathways involved in facilitating HIV infection by the parasite [46].

In pregnant women, the parasite leads to low birth weight and preterm delivery. The latter may be due to the presence of the parasite itself or to treatment with metronidazole [47]. Co-infection with *T. vaginalis* and BV increased IL-1β and IL-8, which in higher levels results in preterm delivery [48,49]. In agreement with these findings, a cohort study evaluated 65 asymptomatic pregnant patients infected with *T. vaginalis*. The concentration of IL-8 and neutrophil defensins were higher in women with asymptomatic trichomoniasis than the uninfected group, confirming the cytokine release and neutrophils activation during trichomoniasis [50]. Furthermore, *T. vaginalis* infection during pregnancy raised serum concentration of granulocyte-macrophage colony-stimulating factor (GM-CSF), which stimulated the production of immune cells such as macrophages, dendritic cells and granulocytes as well as C-reactive protein (CRP), indicating an inflammatory response in systemic levels [51].

The symbiosis between *T. vaginalis* and *M. hominis* may play a role in the clinical complication seen in trichomoniasis during pregnancy. After treatment with metronidazole, large amounts of bacteria were released in the extracellular environment. Once free in this environment, adhesion and infection of human cells occurs [52]. Another study found a prevalence of 9,5% of this bacterial microorganism, being cited as one of the causes of intra-amniotic infection, which can culminate in preterm birth [53].

The association between *T. vaginalis* and prostate cancer remains under debate, while some studies have demonstrated the mechanism involved in signaling tumor-like cells, others have not found epidemiological evidence [54,55]. Due to the focus of this review, the topic below will discuss the immunological mechanisms described for this association.

Epithelial mesenchymal transition (EMT) is the transformation of epithelial cells into stromal cells, being related to tumor progression and has been associated with the

NF-kB and JAK/STAT3 activation. In this context, human prostate epithelial cells co-incubated with live *T. vaginalis* increased CCL2 expression, but not its production, while CXCL8, IL-1β, IL-6 enhanced in both protein and mRNA levels. This interaction induced EMT via NF-kB and JAK/STAT signal pathway, while an increased in H_2O_2 generation via the NADPH oxidase system activated ERK and NF-kB, producing IL-1β and CXCL8, leading to monocyte migration. IL-6 via IL-6R/JAK2/STAT3 stimulates M2 macrophages polarization that accumulate in prostate tumor tissue, contributing to the proliferation and invasiveness of prostate cancer cells [56-58]. *In vivo*, prostate cancer cells from PC3 lineage cells with *T. vaginalis*-contained medium (TCM) injected into the PC3 xenograft mouse model resulted in an enhancement of the tumor weight and volume as well as EMT-related markers, proliferating cell nuclear antigen (PCNA) and cyclin D1, proliferative signal molecules [59]. Interestingly, adipocytes also can enhance prostate cancer development. Co-incubation with mouse prostate cancer cells with TCM, led adipocytes to increase IL-4, CCL2, IL-6 production, besides mRNAs CCL2, IL-4, IL-13, IL-6. In turn, IL-4 and IL-13 stimulated macrophage migration and polarization to M2 macrophages [60]. Prostate stromal cells infected with trichomonads recruited neutrophils and monocytes by CXCL8, CCL2 released by the activation of TLR4, ROS, MAPK and NF-kB and stimulated the proliferation of benign prostatic hyperplasia cells and EMT by IL-6. Then, *T. vaginalis*-stimulated benign prostatic hyperplasia epithelial cells increased IL-6, CXCL8, CCL2 and IL-1β, resulting in monocytes and mast cells migration, as well as mast cells activation. In turn, mast cells increased the prostate stromal cells proliferation that showed an enhancement of cyclin D1 and B-cell lymphoma-2 (Bcl-2) expression [61-64]. In agreement, mouse cells demonstrated the same behavior. Mouse prostate cancer (PCa) cells in contact with *T. vaginalis* recruited mouse macrophages through the production of CCL2 and CXCL1, which in turn,

increased the production and mRNA CCL2, IL-6 and TNF-α expression as well as the invasiveness of PCa cells and the cytokine receptors GP130, CCR2, and CXCR2 expression, positively affecting the proliferation and invasiveness of these cells [65]. In vivo, when the mouse prostate was injected with a mixture of trichomonads and PCa cells, it was observed an increase in EMT and Cyclin D1 as well as larger prostate cancer than demonstrated in group injection of only PCa cells [66].

Finally, *T. vaginalis* produced TvMIF, a human cytokine homolog that mimics the HuMIF and stimulated human monocytes to produce IL-8, contributing to the inflammatory environment and interacting with CD74 receptors. The latter leads to downstream of ERK and Akt/BAD activation, an anti-apoptotic pathway, increasing the tumor growth and cell invasiveness. Interestingly, TvMIF was frequently found in patients with positive rather than negative infection, suggesting there is a TvMIF release in the extracellular environment *in vivo*. In addition, it has been shown to be more present in men, either because of the stronger immune response in men against TvMIF or because of the greater release by the parasite in men than in women [67].

The long permanence of *T. vaginalis* can cause several clinical complications. In this topic, three of them were highlighted: the increase in HIV transmission and acquisition, due to the disruption of the mucosal barrier, reduction of SLPI and recruitment of $CD4^{+}$T cells; adverse pregnancy outcomes caused by a systemic inflammatory response with cytokine production, neutrophils migration and *M. hominis* release into the extracellular environment and prostate cancer attributed to a sequence of inflammatory mechanisms that raised the invasiveness and growth of prostate cells, creating a perfect microenvironment for the establishment of cancer.

3. The infinity stones: Vaccine candidate targets

Some proteins of *Trichomonas vaginalis* play an essential role in the establishment of infection and in the parasite-host relationship, being, therefore, promising vaccine candidate targets.

Adhesion protein (AP) 65-1, involved the cytoadherence, was localized in the cytoplasm and on the surface of the parasite membrane and conferred protective effects in rodents. An increase in time and in the survival rate was observed in the immunized group, characterized by IgG production with a higher expression of IgG1 than IgG2a and a high concentration of the cytokines IFN-γ, IL-2 and IL-10, suggesting the involvement of Th1 and Th2 type cells [68,69]. Likewise, TvAP33 was also detected on most of the trichomonad surface. When inoculated into mice, it showed an enhancement in the survival rate in the immunized groups, with raise of IL-4, IL-10 and IL-17 cytokines and IgG, IgG1 and IgG2a production. It was concluded that there was cellular participation of the Th2 type due to higher levels of IgG1 than IgG2a [70].

Cysteine protease (CP) 30 was found in all the 20 fresh isolates from symptomatic and 20 asymptomatic patients investigated in India. Higher intensity band and cytoadherence to vaginal epithelial cells were detected in isolates from symptomatic than asymptomatic, while antibodies anti-CP30 affected more the cytoadherence in the symptomatic isolate, suggesting that the expression of CP30, adherence and colonization could be higher in symptomatic isolates than asymptomatic isolates [71]. The group of BALB/c mice immunized with intranasal injection of 62kDa cysteine-proteinase (p-62) plus cholera toxin (CT) enhanced the IgG1, IgG2a, IgG3 sera circulating levels and IgG and high IgA levels specific to p-62 in vaginal secretion. It was observed similar results in vaginal secretion when p-62 plus CpG-oligodeoxynucleotides (CpG) immunization was tested. However, in sera there was an increase of IgG2a, IgG2b and IgG3 [72]. A few years later, *T. vaginalis* anti-protease monoclonal antibodies (MAbs)

named 4D8 and 1A8 were tested against the same protease. As result, the epitopes demonstrated a repetitive nature and after treatments with pronase, heat and TCA, the reactivity was reduced. In conclusion, 4D8 obtained a better protective activity in murine model than 1A8, which can be explained by the interaction of MABs with different protein epitopes [73]. 4D8 and 1A8 also inhibited cytoadherence in the HeLa cells. However, 62kDa anti-protease MAbs in the presence of macrophages increased the NO levels. *In vivo*, the group of rodents vaccinated with 4D8 demonstrated higher protection against lesions than other groups, while NO levels in mice serum were present in both tested groups, suggesting that antibodies can induce cytotoxicity mechanisms against the parasite [74].

Two peptides from α-actinin: ACT-F and ACT-T were used in high dosages as immunization. ACT-T conferred a protection of 100% to the mice, while ACT-F high dosage only protected 55% of the animals. High amounts of IFN-γ, IL-17A and IL-6 cytokines and barely levels of IL-2 and IL-4 were detected in both tested groups. In addition, both peptides produced high levels of specific IgG, in special IgG1 which was in greater concentration than IgG2. However, IL-10 was higher in the ACT-T group than ACT-F group, suggesting a Th1/Th2 response [75]. The transient receptor potential-like channel of *T. vaginalis* (TvTRPV) was also tested as a vaccine candidate target. In high doses, a raised immune response in BALB/c mice with high IgG production in sera was observed. In addition, TvTRPV can be recognized by antigen-presenting in macrophages which stimulated the IL-1β, IL-6 and TNF-α production. $CD4^{+}$T cells incubated with mouse macrophages and stimulated with TvTRPV produced detectable levels of IL-10 and IFN-γ that increased the cytotoxicity of macrophages against the protozoan, facilitating its elimination [76].

The unique N-terminal from enolases, a group of enzymes which participate in the glycolysis and play a role of plasminogen receptor, also was described as a potential target against *T. vaginalis*. However, in this study there was no development or test as an immunizing agent [77]. Finally, whole *T. vaginalis* cells were proposed as immunization strategy. Mice group that received the vaccine candidate target had higher IgG and IgG1 serum total levels than the unimmunized group. The production of IgG2a was variable between the tested groups [44].

One of the proposed ways to combat trichomoniasis is through immunization. However, due to currently lack of an adequate animal model for *T. vaginalis* infection, this becomes an arduous task. The most used are mice with an estrogen treatment that compromises the immune response, making it difficult and inaccurate to evaluate the humoral and cellular responses which negatively impact in the vaccine development against this parasite [77]. Table 1 summarizes the vaccine candidate targets and their immune system activation discussed in this topic.

Table 1. Vaccine candidate targets against trichomoniasis

Vaccine candidate targets	Antibody response	Cytokine response	Ref.
AP 65	**IgG; (IgG1 > IgG2a)**	**IFN-γ, IL-2 and IL-10**	**[69]**
AP 33	**IgG; (IgG1 > IgG2a)**	**IL-4, IL-10 and IL-17**	**[70]**
CP 30	**Not mentioned**	**Not mentioned**	**[71]**
62 kDa proteinase + cholera toxin	**IgG1, IgG2a, IgG3 (sera); IgA and IgG (vaginal secretion)**	**Not mentioned**	**[72]**
62 kDa proteinase + CpG-oligodeoxynucleotides	**IgG2a, IgG2b and IgG3 (sera); IgA and IgG (vaginal secretion)**	**Not mentioned**	**[72]**
ACT-T	**IgG; (IgG1 > IgG2a)**	**IFN-γ, IL-17A, IL-6, IL-10**	**[75]**

ACT-F	**IgG; (IgG1 > IgG2a)**	**IFN-γ, IL-17A, IL-6, IL-10**	**[75]**
TvTRPV	**IgG**	**IL-1β, IL-6, TNF-α, IL-10 and IFN-γ**	**[76]**
N-terminal from enolases	**Not mentioned**	**Not mentioned**	**[77]**
***T. vaginalis* whole cells**	**IgG and IgG1 with variable IgG2a**	**Not mentioned**	**[44]**

4. Hope in the midst of war: Antibody production during trichomoniasis

During a *T. vaginalis* infection, there are an antibodies production. Although they are not protective against reinfection, they may be associated with the presence of symptoms or the parasite itself. In this context, some authors have tried to find an association between certain antibodies and symptoms.

Through the mouse model, a study was performed in order to evaluate the circulating antibodies during infection caused by *T. vaginalis*. In vaginal washes and serum, IgA, IL-2, TNF-α, $CD3^+$ T cells, $CD4^+$ T cells and NK cells were found at higher levels in asymptomatic than symptomatic patients [79]. In a fourteen days post infection, IgG, IgG1, and IgM levels were higher in serum samples of rodents infected with isolates from symptomatic patients than isolates from asymptomatic [80]. In agreement, when antibodies were researching in serum of symptomatic patients, IgG, IgG1, IgG2 and IgA were detected in 100% of cases, while IgG, IgG2 and IgA were present in 25% of cases, with no detection of IgG1 in asymptomatic patients. In vaginal washes, IgM was not detected in symptomatic patients, while IgG was detected in all patients tested [81].

Finally, the antibodies production was associated with a protection to adverse outcomes of pregnancy (AOP) and therapy successful. Pregnant women *T. vaginalis*-positive with no AOP had higher IgG anti-TvLPS in vaginal secretion than the group with AOP [82]. After effective therapy with metronidazole, a diminished circulating IgG anti-*T.*

vaginalis was detected, suggesting that there was no longer antigenic stimulation. However, in a few patients which had a treatment failure, reinfection or an infection by resistant isolates, the persistence of antibody was detected which can be associated with a chronic infection [83].

In conclusion, IgG and IgG1 was found in serum samples of rodents infected with isolates from symptomatic patients as well as patients with symptoms. However, this antibodies production does not protect against the establishment of *T. vaginalis* infection. The association between symptoms and the presence of antibodies remains under investigation.

5. An extra help: *M. hominis* and *T. vaginalis* against host immune response

The endosymbiosis between *M. hominis* and *T. vaginalis* have gained prominence in recent years. Several articles demonstrated that the bacteria can survive within the parasite cytoplasmic vacuoles and its presence is capable of influencing the *T. vaginalis* biochemical pathways, tumorigenesis, outcomes in the pregnancy and immunomodulation. Thus, this association can impact in the immune response, survival and outcomes observed during trichomoniasis [84,85].

This interaction increased the mycoplasmal drug resistance and protects the *M. hominis* from antibiotics. When the cultures were treated with gentamicin, the bacterial DNA was detected, suggesting a replication inside the parasite which was reinforced by the found of 5-Bromodeoxyuridine (5-BrdU) incorporated in trichomonad DNA, which might affected PCR-based analytical methods, golden pattern of trichomoniasis detection [86-88]. On the other hand, the effects on metronidazole resistance are under debate. Some authors defends that *T. vaginalis* resistance to MTZ cannot be attributed by *M. hominis* presence [89-92], while other speculate that bacterial presence played a role in

related expression levels with resistance, such as PFOR and demonstrated that 7 of 8 resistant isolates were *M. hominis* positive [93,94]. Furthermore, it is possible that this endosymbiosis may actually reduce resistance to metronidazole, making the isolate more susceptible and suggesting that downregulation of PFOR was not necessarily related to drug resistance [95].

The difference among *T. vaginalis* isolates appeared to be related with the number of infecting bacteria found, although this endosymbiosis was not observed in all *T. vaginalis* isolates [87]. Interestingly, *Mycoplasma*-positive *T. vaginalis* isolates can infect both *Mycoplasma*-free *T. vaginalis* isolates and human cells, while a single *T. vaginalis* isolate can be infected by two mycoplasma species [95,96]. Recent studies have shown that *T. vaginalis* can be infected not only by *M. hominis*, but also by *Candidatus* Mycoplasma girerdii, previously known as Mnola which demonstrated 78% similarity with *M. hominis* and 85% with *M. genitalium,* and another unknown *Mycoplasma* spp. In addition, the hypothesis that the protozoan may have symbiosis with other bacteria, such as *Ureaplasma* spp., needs further studies [85,97]. In the presence of any *Mycoplasma* species, the parasite demonstrated an upregulation of hemolysis and binding to host epithelial cells, indicating a bacterial influence on the virulence observed in the *T. vaginalis* isolates [98].

M. hominis and *T. vaginalis* also impacts in the immune response. The presence of both microorganisms activated the NF-kB, upregulated the expression of IL-8, TNF-α, IL-1β and IL-23 by human macrophages and increased IL-6 levels as compared with no mycoplasma presence [99,100]. In addition, the bacteria can influence the growth rate of *T. vaginalis*, being ~20% faster in the presence of the symbiont. Furthermore, a competition between macrophages and bacteria for arginine uptake leads to downregulation of NO production in the mononuclear cells [100]. Nevertheless, this

combination potentiated the cytopathic effects on epithelial cells with reduced viability and increased in the intercellular spaces, suggesting a virulence enhancement, since a higher rate of amoeboid transformation and phagocytic activity was observed. However, no impact on the leukocytotoxic effects caused by *T. vaginalis* was observed [101,102]. Interestingly, PBMCs in contact with small extracellular vesicles from isolates with *T. vaginalis* virus inhibited the IL-8 response to the signaling of *Mycoplasma*-derived macrophage-activating lipopeptide-2 (MALP-2), a mycoplasma protein, reducing the immune detection of the bacteria [103].

Trichomonas vaginalis and *Mycoplasma* species have a beneficial relationship for both sides. For the bacteria, the parasite works as a “trojan horse” with extra protection against immune defense and antibiotics in the extracellular environment, while for the parasite there is an increase in the growth rate, virulence, and modulation of the immune response, allowing its survival under stress condition. Further studies are needed on the possibility of *T. vaginalis* carrying other microorganisms, such as *Ureaplasma* spp.

Conclusion

In this study it was demonstrated that the parasite can activate and stimulate variated and complex immunological mechanisms that may be related to the symptoms and clinical complications observed. The symbiosis with *M. hominis* also deserves further studies to understand the extent of this relationship. As a conclusion of this review, the war between *T. vaginalis* and host immune system is far from over. More studies are needed in the immunological field to understand the protozoan behavior, aiming at new targets to fight this infection.

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ANEXOS

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Acknowledgments

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2. Casillas I and Buffon GL (**2008**). Rasputinine promotes mating and fertilization efficiency in model organisms. **Microb Cell** 3(4): 23-34. doi: 69.3319/micc.2008.00011

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– Books; examples:

1 1. Salt P (**2010**). Unicellular models for multicellular applications. 3rd edition. **Shared Science Publishers, Graz**.

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– Book Chapters; example:

1. Pepper S (**2006**). Flow cytometry in yeast research. In: Harden XY, Soften AB, editors. The power of flow cytometry. **Shared Science Publishers, Graz**; pp 43-57.

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