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Impact of *T. Vaginalis* Infection on Innate Immune Responses and Reproductive Outcome

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Abstract

Trichomonas vaginalis is the most common non-viral sexually transmitted pathogen. The infection is prevalent in reproductive age women and is associated with vaginitis, endometritis, adnexitis, pyosalpinx, infertility, preterm birth, low birth weight, bacterial vaginosis, and increased risk of cervical cancer, HPV, and HIV infection. In men, its complications include urethritis, prostatitis, epididymitis, and infertility through inflammatory damage or interference with the sperm function. The infection is often asymptomatic and recurrent despite the presence of specific antibodies, suggesting the importance of the innate immune defense. *T. vaginalis* adhesion proteins, cysteine proteases, and the major parasite lipophosphoglycan (LPG) play distinct roles in the pathogenesis and evasion of host immunity. LPG plays a key role in the parasite adherence and signaling to human vaginal and cervical epithelial cells, which is at least in part mediated by galectins. The epithelial cells respond to *T. vaginalis* infection and purified LPG by selective upregulation of proinflammatory mediators. At the same time, *T. vaginalis* triggers an immunosuppressive response in monocytes, macrophages, and dendritic cells. The molecular mechanisms underlying reproductive complications and epidemiologic risks associated with *T. vaginalis* infection remain to be elucidated.

Keywords

Trichomonas vaginalis; lipophosphoglycan; cytokines; galectins; human vaginal epithelial cells

1. Unique features of *T. vaginalis*: more than 170 years of history

T. vaginalis is a sexually transmitted extracellular flagellated single-cell parasite that lives in the female lower reproductive tract and the male urethra. Unique genetic and structural features place the parasite at the base of the eukaryotic phylogenetic tree and suggest an intriguing evolution toward mucosal parasitism. *T. vaginalis* selectively adheres to the human vaginal epithelial cells, surviving for years in the hostile vaginal environment that is typically acidic, contains a plethora of microbicidal innate immune factors, and is reinforced by the presence of a complex commensal bacterial consortium.

T. vaginalis was first recorded in the medical literature more than 170 years ago by a European physician, Alfred Done, who described the trichomonad in human vaginal discharge in 1836.

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It was then regarded as a harmless inhabitant of the vagina for nearly 80 years until linked to vaginitis (for a more comprehensive review, see (Singh et al., 2007)). Since then the parasite's morphology and life cycle have been extensively studied, but still little is known about the immunopathogenesis of trichomoniasis and the molecular mechanisms exploited by *T. vaginalis* to evade the immune system. Some unique features of the parasite include: a huge genome (176,441,227 bp), which is one of the most repetitive known genomes (65%) speculated to emerge from a transition from enteric to urogenital tract environment; utilization of hydrogenosomes instead of mitochondria, which in contrast to mitochondria contain no genetic material; a rare mix of enzymes from both eukaryotic and prokaryotic class; and a unique lipophosphoglycan glycosyl-phosphatidylinositol (GPI) molecule, which has shown compositional similarities with prokaryotic glycoconjugates and plays a major pathogenic and immunoregulatory role (Carlton et al., 2007; Fichorova et al., 2006; Huang et al., 2009; Singh et al., 2009; Vanacova et al., 2003; Zubáková et al., 2008). In addition, *T. vaginalis* carries viruses and mycoplasma that may be linked to severity of mucosal damage, inflammatory symptoms, and consequences for reproductive outcome (Alderete et al., 1986).

2. Clinical manifestations and impact on reproductive health

In women *T. vaginalis* causes a wide spectrum of symptoms, ranging from a relatively asymptomatic state to severe inflammation and irritation with foul-smelling vaginal discharge, low abdominal pain, and dysuria. The associated morbidities and complications include vaginitis, endometritis, adnexitis, pyosalpinx, atypical pelvic inflammatory disease, preterm birth, premature membrane rupture, low birth weight, infertility, cervical cancer, enhanced risk of HIV-1 and other viral infections, and a positive association with bacterial vaginosis (McClelland, 2008; Shafir et al., 2009; Singh et al., 2007; Weinstock et al., 2004). Female infants can get infected during birth; infection may remain asymptomatic until puberty (Smith et al., 2002).

T. vaginalis has been detected in 66–77% of the male partners of infected women, and of those men, about 70% were asymptomatic (Sena et al., 2007). In men the infection, although usually self-limiting and often asymptomatic, is associated with urethritis, prostatitis, epididymitis, reduced sperm function, and infertility (reviewed in (Singh et al., 2007) and (Benchimol et al., 2008)).

It is important to note that the *T. vaginalis* infection is often recurrent, with no lasting immunity, suggesting the importance of innate immunity. The choice of treatment since 1960 has been metronidazole; however, resistance to this drug is increasing (Shafir et al., 2009). In addition, the metronidazole treatment is correlated with increased cases of pancreatitis in patients with recurrent trichomoniasis, and metronidazole treatment of symptomatic carriers increases the incidence of preterm birth (Okun et al., 2005).

3. Alarming epidemiologic trends

Worldwide 160 million to 180 million people are infected with *T. vaginalis* each year, and of those 154 million are in resource-limited settings; 8 million to 10 million are in the United States and 11 million are in Europe (McClelland, 2008). The incidence of *T. vaginalis* infection occurs more than Chlamydia and gonorrhea put together. In the United States the prevalence among women ranges from 2.8% in adolescents nationwide to 51% in some black communities (Shafir et al., 2009). In men statistics is less reliable although high prevalence (58%) has been reported in young inner city black men (Shafir et al., 2009). Worldwide estimates may be “underestimates” since they have been largely based on wet mount microscopy, which has 35–82% sensitivity as compared to a more sensitive culture technique or PCR diagnostics (Caliendo et al., 2005; Shafir et al., 2009). Also, current statistics may not represent the general population, as infection is asymptomatic in at least one-third of women and most men. An

alarming fact is the high prevalence of trichomoniasis recorded in otherwise healthy asymptomatic women: one-fifth of asymptomatic women attending Family Planning clinic in South Africa (Johnson et al., 2005) and 47% in the general population attending an obstetrics and gynecology clinic in India (Yadav et al., 2005). Hence, trichomoniasis is the most common non-viral sexually transmitted infection and has reached the level of a global epidemic.

The public health importance of the problem is underscored by the fact that the trichomonas infection is prevalent in reproductive age women with serious adverse reproductive outcomes (McClelland, 2008). The infection is on the rise in adolescents (Van Der Pol et al., 2005; Weinstock et al., 2004).

4. Immunoinflammatory responses to *T. vaginalis* infection in pregnant women

The immunoinflammatory response to trichomoniasis has been most studied in pregnant women. *T. vaginalis* positive pregnant women with bacterial vaginosis (Nugent 7–10) had increased vaginal IL-1 β and neutrophils compared with bacterial vaginosis alone (Cauci and Culhane, 2007). Increased cervical IL-8 and alpha-defensin have been found in pregnant infected women (Simhan et al., 2007). At the same time in symptomatic women, anti-inflammatory mediators such as the soluble leukocyte protease inhibitor (SLPI) were lower (possibly due to digestion by trichomonas cysteine proteases) and reactive nitrogen intermediates were higher (Al-Mohammed and Hussein, 2006; Draper et al., 1998). The presence of increased C-reactive protein in the sera of *T. vaginalis*-infected pregnant women suggests that the impact of the immunoinflammatory reaction to the parasite exceeds the boundaries of the reproductive tract mucosa (Simhan et al., 2007). Mechanisms of pregnancy complications linked to *T. vaginalis* remain elusive. The complex interactions between *T. vaginalis*, the vaginal microflora and the host may hold the key to these complications and must be addressed by future clinical and experimental investigations.

5. Modeling the infection and immunity *in vivo* and *in vitro*

The immunoinflammatory responses to *T. vaginalis* infection have been studied *in vitro* and in mouse models. *In vitro* experimentation has been conducted with cervical and vaginal epithelial cells (Bastida-Corcuera et al., 2005; Fichorova et al., 2006; Kucknoor et al., 2005a; Kucknoor et al., 2005b; Kucknoor et al., 2007; Okumura et al., 2008; Singh et al., 2009; Sommer et al., 2005), and with various immune cell types (Chang et al., 2006a; Chang et al., 2004; Ryu et al., 2004; Shaio et al., 1995). Both proinflammatory and immunosuppressive responses have been observed as discussed below.

Mice challenged with asymptomatic compared with symptomatic *T. vaginalis* isolates responded by increased IgA, IgG, Th1 cytokines, and production of reactive nitrogen intermediates (Malla et al., 2007). Both circulating and mucosal antibodies have been detected in women (Kaur et al., 2008); however, the immunity is not lasting, and it has been shown that *T. vaginalis* cysteine proteases present in the serum and vaginal secretions of symptomatic women can degrade IgG, IgM, and IgA (Yadav et al., 2007). In addition, the *T. vaginalis* cysteine proteases including CP30, induce apoptosis in vaginal epithelial cells (Sommer et al., 2005) and in multiple mucosal immune cell types (discussed below).

In leukocyte culture, *T. vaginalis* stimulated IL-8, leukotrienes, reactive nitrogen intermediates, and inducible nitric oxide synthase (iNOS) (Ryu et al., 2004; Shaio et al., 1995). *T. vaginalis* has been shown to produce leukotrienes capable of activating the host cells (Shaio et al., 1992). In T cells, macrophages, and dendritic cells, *T. vaginalis* led to apoptosis and production of immunosuppressive cytokines (IL-10, TGF β) (Chang et al., 2004). *T. vaginalis* proteins

(adhesins and CP30) induce caspase-mediated apoptosis and immunosuppressive cytokine response (Chang et al., 2006a). *T. vaginalis*-induced apoptosis in neutrophils has been linked to caspase-3 activation and reduced expression of the anti-apoptotic protein myeloid cell leukemia sequence 1 (Mcl-1) (Kang et al., 2006), and in macrophages it has been linked to extracellular signal-regulated kinases (ERK) activation (Chang et al., 2006b). *T. vaginalis* infection has been shown to activate toll-like receptors (TLR)-4 by inducing undefined substance(s) released in the vaginal secretions (Zariffard et al., 2004). To date, trichomonad ligands for TLR4 have not been identified. *T. vaginalis* has been reported to induce COX-2 expression and TLR2, 4, and 9 upregulation via the p38 mitogen-activated protein kinase (MAPK) pathway (Chang et al., 2006a). Host receptors mediating these effects remain elusive.

The human cervical and vaginal epithelial cells represent the most physiologic model for the study of the *T. vaginalis* infection because they are the host cells supporting the parasite adherence, survival, and replication in the genital tract. Immortalized human vaginal and cervical epithelial cell lines have been generated and used for modeling the epithelial immune response and inflammatory reaction (Fichorova et al., 1997). This experimental model has yielded important information on the species specificity of *T. vaginalis*-induced infection and the molecular mechanisms of host-parasite interaction discussed below (Bastida-Corcuera et al., 2005; Fichorova et al., 2006; Okumura et al., 2008; Singh et al., 2009). *T. vaginalis* adhesion to the human vaginal epithelial cells is species specific and capable of inducing gene upregulation not only in the host (Fichorova et al., 2006; Kucknoor et al., 2005a) but also in the parasite (Kucknoor et al., 2005b).

6. The *T. vaginalis* lipophosphoglycan – functional domains and innate immunity mechanisms

The *T. vaginalis* lipophosphoglycan (LPG) has become the focus of extensive studies ever since it was discovered that it is the most abundant glycoconjugate on the parasite surface ($2-3 \times 10^6$ copies/parasite) and is responsible for the parasite adherence to the vaginal epithelial cells (Singh et al., 2007). It is a pure carbolipid (no peptide component) anchored on the parasite surface via inositol-phosphoceramide. Unlike LPG from other parasites (*Leishmania*), *Trichomonas* LPG does not undergo structural changes during parasite development (Singh et al., 2007). A dose-response relationship between *T. vaginalis* LPG and the parasite adherence has been observed (Fichorova et al., 2006). LPG-truncated mutants express reduced adherence to human ectocervical epithelial cells (Bastida-Corcuera et al., 2005). *T. vaginalis* LPG triggers species-specific inflammatory response and selective chemokine (IL-8 and macrophage inflammatory protein-3 α) upregulation by human cervical and vaginal epithelial cells in a TLR-4 independent fashion (Fichorova et al., 2006).

The structure and composition of *T. vaginalis* LPG have been recently revealed and specific LPG domains have been linked to its proinflammatory properties (Singh et al., 2009). Mild acid and consecutive enzymatic treatment has led to the isolation and characterization of the outer branch saccharide and the ceramide phospho-inositol glycan core (CPI-GC) of *T. vaginalis* LPG. It was determined that LPG signals via its CPI-GC core since CPI-GC, with purity confirmed by mass spectrometry, induced a significant upregulation of IL-8 at the protein and mRNA level at concentrations equivalent to multiplicity of infection of 1–4 parasites per epithelial cells. LPG and its CPI-GC core activated NF κ B, ERK1/2, and MEK1/2 (Singh et al., 2009).

Recent comprehensive compositional and structural analysis of *T. vaginalis* revealed that LPG CPI-GC contains terminal poly-N-acetyllactosamine repeats that represent the ligand for the animal lectins called galectins (Singh et al., 2009). It has been observed that galectins-1 and -3 are expressed by the normal human cervical and vaginal epithelial cells and are capable of

binding purified LPG (Fichorova et al., 2008). A recent study showed that galectin-1 mediates adherence to cervical epithelial cells (Okumura et al., 2008). *T. vaginalis* chemical mutants with truncated LPG (reduced Gal and GlcN) showed reduced binding to cervical epithelial cells compared with wild-type parasites. Exogenous galectin-1 enhanced adhesion to cervical epithelial cells. The cross-linking properties of the dimeric galectin-1 form contributes to high avidity *T. vaginalis* binding (Okumura et al., 2008). Vaginal epithelial cells released galectin-1 and galectin-3 upon *T. vaginalis* infection, and these galectins modulated the vaginal epithelial cell inflammatory responses (Fichorova et al., 2008).

The galectins represent an evolutionarily conserved family of glycoproteins that contain one or two carbohydrate-recognition domains with affinity for galactose-containing oligosaccharides (β -galactosides) (Rabinovich and Gruppi, 2005). Galectin-3 is unique in that it contains one carbohydrate-recognition domain and one non-carbohydrate-recognition domain binding site. The intracellular galectins (such as galectin-3) regulate pre-mRNA splicing transported to the nucleus for transcriptional regulation. Galectins can also be membrane-bound or secreted, and on the cell surface they cross-link glycoconjugates involved in cell adhesion, proliferation, apoptosis, and multiple immune functions (Rabinovich and Gruppi, 2005). Galectin-1 and galectin-3 have been reported to have opposing immune functions, galectin-1 suppressing and galectin-3 enhancing leukocyte responses to inflammatory stimuli (Nieminen et al., 2008; Rabinovich and Gruppi, 2005).

Galectin-1 and galectin-3 play distinct roles in HIV infection. Galectin-1 promotes viral attachment (Mercier et al., 2008). HIV-1 alters cell surface glycosylation, which promotes galectin-1 binding (Lanteri et al., 2003). HIV-1 Tat, which is essential for viral replication and regulates cytokine expression, enhances galectin-3 expression in infected cells (Fogel et al., 1999). Multiple members of the galectin family have been recently implicated in the female reproductive function (Than et al., 2008a; Than et al., 2008b). Thus, the *T. vaginalis* LPG interaction with galectins may be part of the molecular mechanisms of increased risk of HIV-1 infection and reproductive problems associated with trichomoniasis.

8. Future directions

Future studies are needed to identify the host receptors and the host's as well as parasite's pathways involved in the symptomatic and asymptomatic sequelae and immune evasion by *T. vaginalis*. The mechanisms of the synergistic interactions between *T. vaginalis* and bacterial vaginosis, the most common vaginal syndrome of reproductive age women, are virtually unknown. More research is needed to understand and prevent the negative impact of *T. vaginalis* on women's reproductive health and the risk of other sexually transmitted diseases.

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