Trichomonas vaginalis: Lifestyle, Cellular Biology, and Molecular Mechanisms of Pathogenesis

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Abstract *Trichomonas vaginalis* is a unicellular, flagellated, microaerophilic protozoan that colonizes the human urogenital tract extracellularly. It causes trichomoniasis, a highly prevalent sexually-transmitted infection (STI). In this chapter, we will give a brief description of disease outcomes of *T. vaginalis* infection, and then discuss the current understanding of the parasite biology and pathogenic mechanisms. Of note, *T. vaginalis* pathobiology occurs in the presence of the vaginal microbiota, and often with at least one of several parasite symbionts, which we will also discuss to give a more holistic picture. Throughout, we will highlight both noteworthy historical, as well as exciting recent research that has shaped our understanding of *T. vaginalis*, and point out where technological advances have enabled a deeper understanding of the parasite. We close with a discussion of treatment strategies that *T. vaginalis* research has informed, and future perspectives for research avenues that will both aid in improved management of trichomoniasis, and develop a more complete understanding of the life of this fascinating organism.

1 Part 1: Introduction

With a global prevalence approaching 400 million (Fiori et al. 2013; WHO 2018) and annual new infections globally reported to be 156 million (WHO 2018), trichomoniasis is the third-most common sexually transmitted infection, highlighting the potent ability of *T. vaginalis* to colonize its human host. Trichomoniasis prevalence is only exceeded by HPV (Human Papilloma Virus) and HSV-2 (Herpes Simplex Virus Type 2, genital herpes). Discovered in 1836, *T. vaginalis* was historically considered to be a member of the commensal vaginal microbiome, however its pathogenic properties began to be appreciated about a century later. Still, the complexity of trichomoniasis is not well understood, with reports of symptoms ranging from none, to moderate, to severe, putatively due to variation in (1) human immune responses, (2) indigenous vaginal microbes, and (3) the inherent virulence of the strain, including whether the strain harbors any microbial symbionts (Mercer and Johnson 2018). From a cell biology perspective, *T. vaginalis* flagella and cytoskeletal elements are some of the parasite's most visually strinking features and poised to reveal unique *T. vaginalis* biology. Various biochemical processes in the parasite, in addition to promoting survival within a unique

compartment in the human host, also likely intersect with pathogenesis. Studies have zeroed in on particular molecular players and cellular pathways that contribute to *T. vaginalis* interactions with host cells. Adhesins, proteases, secreted vesicles, regulatory gene expression, and signaling events help mediate *T. vaginalis* adhesive properties and its cytotoxic effects. It is now important to investigate how *T. vaginalis* employs these factors and pathways during an in vivo infection. The latter is also needed to fully understand the parasite's impact on the surrounding host microenvironment including effects on promoting inflammation and modulation of the urogenital microbiome. In this chapter we will delve deeper into these multifaceted aspects of the *T. vaginalis* lifecycle.

2 Part 2: Trichomoniasis; Disease and Outcomes

T. vaginalis can affect both sexes, and most infections are mild or asymptomtic. Below, we outline current knowledge of *T. vaginalis* pathology in women, men, and neonates.

2.1 Effects on Women

The morbidity burden of trichomoniasis is disproportionately carried by women. With an overall prevalence of 1.8%, the United States carries a higher burden than other high-income countries, pointing to the lack of public health attention paid to the parasite in the US, where it is currently classified as a neglected parasitic infection (Secor et al. 2014; Van Gerwen and Muzny 2019). African Americans are disproportionately affected by 17-fold, compared to other ethnic backgrounds (Van Gerwen and Muzny 2019). *T. vaginalis* is also reported to have a prevalence of up to 43% in Human Immunodeficiency Virus (HIV)+ women (Fastring et al. 2014; Van Gerwen and Muzny 2019). Common symptoms include vaginitis and vaginal discharge. However, serious adverse consequences include pelvic inflammatory disease (Wiringa et al. 2019), increased risk of both HIV transmission and acquisition, and increased risk of malignant cervical cancers (RN Fichorova 2009; Mielczarek and Blaszkowska 2016). One study reported a 2.62 odds ratio of high-grade cervical squamous intraepithelial lesions with *T. vaginalis* (Tao et al. 2014), and another showed that among HPV+ women, those also infected with *T. vaginalis* were more likely to have cervical cancer lesions (Ghosh et al. 2017). A recent meta-analysis also upholds this link in all ethnic backgrounds studied, which included European, African, and Asian women (Yang et al. 2018). How *T. vaginalis* contributes to cervical cancer progression remains unknown, but it could be due to inciting

more tissue damage and inflammation in HPV+ neoplasms, which in turn promotes their metastasis.

2.2 Effects on Reproductive Outcome and Neonates

T. vaginalis is associated with female and male infertility, and with pre-term/low-weight infant births (RN Fichorova 2009; Mielczarek and Blaszkowska 2016; Wiringa et al. 2019). *T. vaginalis* prevalence in pregnant women was found to range from 3.9% to 24.6% in low- to middle income countries (Van Gerwen and Muzny 2019). The strongest adverse consequences are thought to be reductions in carrying a fetus to term. However, aside from sequelae of premature birth, *T. vaginalis* is not thought to directly cause disease in neonates. A recent meta-analysis of all reported studies on *T. vaginalis* and pregnancy, upholds a link between *T. vaginalis* and perinatal morbidity (Silver et al. 2014), and a new study in Uganda upholds a specific link between *T. vaginalis* infection and premature uterine membrane rupture among STIs; Chlamydia and HSV-2 had no link (Nakubulwa et al. 2015). Recently, a report also cited a single case of a neonate born with *T. vaginalis*, found in a brain abscess (Hamilton et al. 2018), however this is not a common occurrence.

2.3 Effects on Men

Although most men are asymptomatic and can have high spontaneous clearance rates, *T. vaginalis* can cause inflammation in the prostate (prostatitis) (Van Gerwen and Muzny 2019). Furthermore, *T. vaginalis* sero-positivity was found to be associated with prostate cancer (PC) (Sutcliffe et al. 2006), particularly in cases that are metastatic (Stark et al. 2009). However, more recent studies observing larger cohorts of African American men, who are at increased risk for both *T. vaginalis* and PC, failed to support this correlation (Marous et al. 2017). Another recent study also failed to find a correlation between *T. vaginalis* sero-positivity and metastatic or fatal prostate cancer (Shui et al. 2016), and a recent metanalysis of all correlation studies including *T. vaginalis* and PC performed across many ethnic groups also identified only a modest increase that was not statistically significant (Najafi et al. 2019). Therefore, *T. vaginalis* is unlikely to be a major risk factor for prostate cancer. Intriguingly however, *T. vaginalis* produces a protein called TvMIF, a protein mimic of human Macrophage Migration Inhibitory Factor (MIF), which is associated with PC (Meyer-Siegler et al. 2006, 2005). Researchers also demonstrated that TvMIF could promote prostate cell growth and metastatic behavior in vitro (Twu et al. 2014). Therefore, it is possible that TvMIF could be involved in promotion of some cases of metastatic prostate cancers despite the fact that recent studies fail to uphold signitifcant increases in PC among *T. vaginalis*+ men, since PC is highly multi-

factorial in etiology. Furthermore, TvMIF could promote cell growth and invasion during cervical cancer metastasis. TvMIF also contributes to inflammation (Twu et al. 2014), which is associated with myriad *T. vaginalis*-associated pathologies and also contributes to metastatic processes.

2.4 Transmission

As a sexually-transmitted parasite, *T. vaginalis* can be transmitted through genital contact from male-to-female, from female-to-male, and from female-to-female (Muzny et al. 2012, 2011; Van Gerwen and Muzny 2019). It is less commonly transmitted from male-to-male, but reports of rectal transmission in men who have sex with men are now emerging (Hoffman et al. 2018). While oral sex is not thought to be a major mode of transmission, one case of vaginal-oral-pharyngeal transmission was recently reported (Kitty Carter-Wicker and Omole 2016). Safer-sex practices can reduce transmission, as condoms confer good protection (Crosby et al. 2012). Furthermore, a recent study confirmed old reports that male partner circumcision reduces male-to-female transmission by approximately 50% (Gray et al. 2009; Pintye et al. 2017). However, studies have had variable results in determining whether circumcision decreased the acquisition of *T. vaginalis* by males from females (Mehta et al. 2009; Sobngwi-Tambekou et al. 2009).

2.5 Diversity of Strains

As discussed above, the clinical presentations of *T. vaginalis* can vary greatly. One reason for the variability in disease is thought to be because many different strains of the parasite are circulating in the population, which likely vary in virulence (Lustig et al. 2013; Mercer et al. 2016). Genetic analysis of *T. vaginalis* strains shows that two main types exist (MD Conrad et al. 2012). Types 1 and 2 can be distinguished using a panel of micro-satellites, and exist in equal proportions worldwide (Conrad et al. 2011, 2012, 2013). Type 1 is more likely to harbor a viral symbiont, *Trichomonasvirus* (discussed in Sect. 5.1), and is correlated with increased HIV viral load in *T. vaginalis*-HIV co-infected women (Conrad et al. 2013). *T. vaginalis* is known to divide asexually. However, modes of sexual reproduction are suspected, since comparison of different strains showed evidence of genetic exchange (Conrad et al. 2012). However, sexual reproduction has never been demonstrated in the laboratory (Bradic and Carlton 2018). It is currently unclear whether the parasite undergoes any sexual reproduction during infection, but it is clear that a great amount of diversity has evolved in this parasite throughout its history within the human host.

Having outlined important clinical features of trichomoniasis, we will now discuss some basic biology of this trichomonad, including its morphology and organelles.

3 Part 3: The Cell Structure and Biology of *T. vaginalis*

T. vaginalis exists as an ovoid trophozoite when grown axenically. However, the parasite transforms to an ameboid form upon binding to surfaces of host epithelial cells (Arroyo et al. 1993; Benchimol et al. 2001; Midlej and Benchimol 2010). Transmission electron microscopy (TEM), scanning electron microscopy (SEM), and recent electron tomography with 3D-reconstruction of *T. vaginalis* have allowed detailed observation of *T. vaginalis* morphology and cellular features, which include interesting structures and organelles outlined below, and shown in Fig. 1. While the specific proteins comprising each of these cellular structures and a full understanding of the biogenesis of these organelles are unknown, recent powerful *omics* work presented below has generated datasets bound to illuminate new aspects of *T. vaginalis* cell biology.

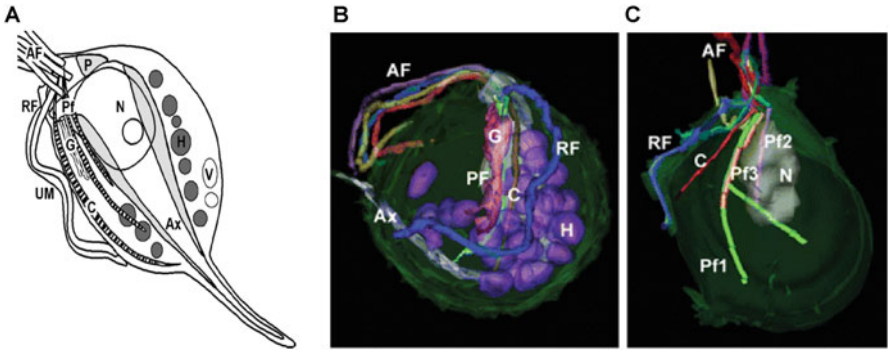

Fig. 1 *T. vaginalis* morphology. (**a**) Schematic diagram of *T. vaginalis.* Anterior flagella (AF), recurrent flagellum (RF), undulating membrane (UM), costa (C), parabasal filament (Pf), pelta (P), nucleus (N), Golgi complex (G), axostyle (Ax), hydrogenosome (H), vacuole (V). (**b, c**) 3D structure of *T. vaginalis.* Using electron tomography, 100-nm sections (**b**) or 250-nm sections (**c**) of *T. vaginalis* were utilized to generate the 3D reconstructions shown. Anterior flagella (AF), axostyle (Ax), costa (C), Golgi complex (G), hydrogenosomes (H), recurrent flagellum (RF), parabasal filaments (Pf 1–3). This figure was reprinted from Copyright (2009) Oxford University Press. Lee et al. Three-dimensional structure of the cytoskeleton in *Trichomonas vaginalis* revealed new features, J Electron Microsc (Tokyo) and publisher

3.1 Structural Features and Dimension

3.1.1 Flagella and Cytoskeleton-Related Structures

T. vaginalis is a motile parasite propelled by flagellar beating (Lee et al. 2009). Five basal bodies (kinetosomes) at the most anterior region of the cell anchor the four anterior flagella and one recurrent flagellum (Lee et al. 2009). The flagellar axonemes have a typical eukaryotic 9 + 2 arrangement of microtubules (Benchimol 2004; Lee et al. 2009). The anterior flagella exits the cell through a flagellar canal that is supported by the crescent-shaped pelta (Benchimol 2004). The pelta also supports the anterior portion of the cell and overlaps with the axostyle in the top part of the cell; this area of overlap is referred to as the pelta-axostyle complex (Marlene Benchimol 2010). The recurrent flagellum exits through another region on the anterior part of the cell and folds downwards towards the posterior of the parasite, remaining attached to the cell body and helping to form the undulating membrane (Benchimol 2010; Benchimol and De Souza 1990; Honigberg et al. 1984). The undulating membrane extends about halfway to three fourths down the *T. vaginalis* cell body (Cheon et al. 2013; Smith and Stewart 1966).

The costa is a unique fiber structure present in trichomonads that contain undulating membranes (Viscogliosi and Brugerolle 1994). The costa emerges from the second basal body, passes underneath the basal body that gives rise to the recurrent flagellum, and continues in the cytoplasm, following the path of the undulating membrane (Lee et al. 2009; Viscogliosi and Brugerolle 1994) (Fig. 1). Due to its adjacent localization to the undulating membrane, the costa is hypothesized to provide structural support to the undulating membrane (Viscogliosi and Brugerolle 1994). Proteins with molecular weights between 100 and 135 kDa are present in the costa-enriched fractions from *T. vaginalis* and other trichomonads (Viscogliosi and Brugerolle 1994). However, the identity of these proteins remains to be determined.

Researchers have developed a methodology to isolate *T. vaginalis* flagella, by physically shearing the parasite and performing sucrose density-gradient centrifugation. Analysis of these isolated flagella also shows the presence of proteins with a wide variety of molecular weights (Jemilohun 1998). However, the complete protein makeup of the *T. vaginalis* flagella is still unknown. Upon exogenous expression of tagged proteins it has been shown that many proteins that localize to the *T. vaginalis* flagella also localize to the plasma membrane. These proteins include two tetraspanin family members TSP1 (TVAG_019180) and TSP6 (TVAG_460770), TVAG_267320 a nicastrin precursor, TVAG_020780 a serine/threonine protein phosphatase, and TVAG_258230, a hypothetical protein (de Miguel et al. 2010, 2012). Some of the identified flagellar proteins also have likely roles in interaction with host cells. For instance, upon co-incubation of ectocervical cells with *T. vaginalis* overexpressing an HA-tagged TSP6 fusion protein (TSP6-HA), TSP6-HA underwent a striking redistribution to the flagella. The C-terminal tail of TSP6 was necessary for flagellar targeting (de Miguel et al. 2012), and when the TSP6 C-terminal tail was fused to two different tetraspanin proteins, it conferred stronger

flagellar targeting of these proteins as well, indicating that *T. vaginalis* contains flagellar-targeting motifs. The concentration of plasma membrane proteins to the flagella upon host cell encounter, together with the identification of other signaling proteins in the flagella, suggest a sensory role for *T. vaginalis* flagella (de Miguel et al. 2012). Interestingly, using freeze-facture electron microscopy, researchers observed the presence of 9-12 intramembranous particles arranged in rosettes on the anterior flagella of *T. vaginalis* (Benchimol and De Souza 1990; Honigberg et al. 1984). In the area where flagella emerge from the parasite, rosettes are also found positioned around the flagella in the form of a "flagellar necklace" (Benchimol 2010). These intriguing flagellar rosettes have also been visualized in other ciliates, but their function similarly remains to be elucidated. It also remains to be investigated whether the five *T. vaginalis* flagella have distinct or complementary roles, which may be revealed as we learn more about their locomotive properties and how these flagella are assembled in the parasite.

3.1.2 Axostyle

Similar to some other protists, *T. vaginalis* also contains an axostyle. The axostyle is a ribbon of longitudinally-oriented microtubules that span from the top to the bottom of the parasite and extends past the end of the cell, forming a small narrow tube that protrudes from the cell posterior (Lee et al. 2009; Ribeiro et al. 2000) (Fig. 1). The axostyle in *T. vaginalis* does not contract and thus is not thought to contribute to motility. Instead, the axostyle helps to maintain the cell axis and has an important role in *T. vaginalis* cell division (Marlene Benchimol 2010), as discussed in Sect. 3.3.

3.1.3 Parabasal Apparatus

As a member of the group Parabasalia, *T. vaginalis* also contains a parabasal apparatus, composed of the Golgi complex (discussed in Sect. 3.1.4) in proximity to parabasal filaments (Benchimol et al. 2001) (Fig. 1a, b). Benchimol et al. observed fibrils connecting the cis-most region of the Golgi to the parabasal filaments and thus the parabasal filaments have been hypothesized to support the Golgi structurally (Benchimol et al. 2001). Recent 3D reconstruction of *T. vaginalis* via electron tomography has now revealed the presence of three parabasal filaments (Lee et al. 2009) instead of two, as previously observed. These filaments arise from the basal bodies and extend downward inside the cell (Benchimol et al. 2001). Parabasal filament 1 splits into two strands and one of these strands then points into the interior of the cell (Fig. 1c). It has also been hypothesized that the twisting structure of parabasal filament 1 may help provide tension and force to aid flagellar beating (Lee et al. 2009), however this model also remains to be tested.

3.1.4 Dimensions

Using scanning electron microscopy, researchers found that the *T. vaginalis* cell body measures 9.5 (7.4–11.4) μm in body length and 6.8 (5.3–7.7) μm in width, and that the total size including the length of the flagella, the body, and the axostyle is 26 (21–32) μm (Cheon et al. 2013). Interestingly, Cheon et al. reported that the axostyle of freshly isolated *T. vaginalis* is longer than that of long-term passaged parasites, measuring 8.2 and 4.0μm, respectively. However, the biologic implication of this finding remains to be investigated.

3.2 Organelles and Metabolism

3.2.1 Golgi Apparatus

As discussed in Sect. 3.1.3, The *T. vaginalis* Golgi has a peculiar association with the parabasal apparatus, a defininig feature of the group Parabasalia. However, many other features of the *T. vaginalis* Golgi are similar to other eukaryotes. It contains 8–12 cisternae (segments), and reaches dimensions of 6μm in length and 1μm in width (Benchimol et al. 2001). Cytochemistry analysis of the *T. vaginalis* Golgi shows the presence of carbohydrates, indicating a role in post-translational glycosylation of proteins, as it does in other eukaryotes (Benchimol et al. 2001). Proteins within the *T. vaginalis* Golgi have been defined, such as AP51 and AP65 (Benchimol et al. 2001), TvROM2 (TVAG_359500), TvROM3 (TVAG_476950) (Riestra et al. 2015), and a BspA protein (TVAG_240680) (Handrich et al. 2019). Some of these proteins have served as Golgi markers (Riestra et al. 2015), indicating specific roles in the organelle, however the majority of the Golgi content and the biochemical reactions that take place there remain to be uncovered.

3.2.2 Endoplasmic Reticulum

T. vaginalis also contains a rough endoplasmic reticulum (ER) system that is located around the nucleus (Smith and Stewart 1966). Along with the Golgi, the ER is predicted to be a site of calcium mobilization, also displaying positive Ca^{++}-ATPase activity (Benchimol 2004), indicating that the Golgi and ER are key regulatory organelles for calcium-dependent cellular signaling. *T. vaginalis* proteins that appear to localize to the ER include the adhesin TVAG_212570 (Handrich et al. 2019) and the tetraspanin protein TvTSP4 (TVAG_458280 (Coceres et al. 2015).

3.2.3 Nucleus and the Mastigont System

The nucleus of *T. vaginalis* is ovoid and is surrounded by a double membrane (Smith and Stewart 1966), and freeze fracture studies have revealed the presence of nuclear pores on the *T. vaginalis* nucleus (Honigberg et al. 1984). Researchers have also observed that the nucleus is closely associated with the mastigont system of *T. vaginalis,* which arises at the *T. vaginalis* cell anterior (Viscogliosi and Brugerolle 1994). The mastigont system refers to kinetosomes and flagellar axonemes, appendages and rootlet filaments that surround the basal bodies, the costa, parabasal filaments, the pelta, and the axostyle (Benchimol 2010). Its close association with the nucleus is important in cell division, as we will discuss in Sect. 3.3.

Other organelles such as hydrogenosomes, lysosomes, and glycogen granules are also present in *T. vaginalis* (Benchimol 2004; Honigberg et al. 1984; Smith and Stewart 1966). Interestingly, hydrogenosomes and glycogen granules are often observed in association with the axostyle (Benchimol 2004). Hydrogenosomes and glycogen granules participate in the parasite's anaerobic metabolism, which we will discuss below.

3.2.4 Hydrogenosomes

T. vaginalis lack mitochondria and instead contain hydrogenosomes. Phylogenetic and biochemical analyses indicate that the two metabolic organelles share a common ancestor (Schneider et al. 2011; Shiflett and Johnson 2010). Similar to mitochondria, hydrogenosomes are double-membrane bound organelles, but unlike mitochondria, they lack a genome. Fermentative metabolism of pyruvate takes place inside hydrogenosomes, leading to the production of ATP. During this process, molecular hydrogen is generated as a by-product, hence the organelle's name (Shiflett and Johnson 2010). Schneider et al. published a proteome of *T. vaginalis* hydrogenosomes, consisting of 569 proteins and identifying putative hydrogenosome biochemical pathways such as amino acid and energy metabolism, iron-sulfur cluster assembly, flavin mediated catalysis, oxygen stress responses and ATP hydrolysis (Schneider et al. 2011). A recently published metabolomic analysis of *T. vaginalis* hydrogenosomes, has provided functional evidence that hydrogenosomes are indeed sites of amino acid metabolism (Huang et al. 2019). The metabolic adaptability of *T. vaginalis* to varying conditions that the parasite might face during infection, particularly low glucose availability and oxidative stress, together with the production of ATP, highlight the vital role of hydrogenosomes in *T. vaginalis* biology.

3.3 Cell Division

In vitro, T. vaginalis doubles approximately every 4–6 h. The parasite undergoes cell division through closed mitosis, a form of mitosis in which the nuclear envelope does not break down. Here, we discuss structures that are important for understanding closed mitosis and describe how the cell morphology changes during cell division.

Prior to mitosis, DNA is replicated and the cell also duplicates the components of the mastigont system (discussed in Sect. 3.2.4), which travel together with the cell bodies during *T. vaginalis* cell division (Benchimol 2004; Ribeiro et al. 2000). A microtubule organizing center (MTOC), also known as an attractophore, appears underneath the second kinetosome in each daughter cell and near the parabasal filament (Benchimol 2010; Bricheux et al. 2007). The attractaphore is a unique morphological feature of parabasalids (Bricheux et al. 2007). Scanning electron microscopy and indirect immunofluorescence assays utilizing anti-tubulin antibodies that recognize the spindle pole, flagella, basal bodies, and the pelta-axostyle together with nuclear staining, have allowed visualization of the movement of these structures during *T. vaginalis* cell division (Bricheux et al. 2007; Ribeiro et al. 2000; Zuo et al. 1999). A summary of these overall findings is described below and shown in Fig. 2.

During interphase and prophase, the attractophore, is initially observed as a round "dot-like" structure next to one pole of the nucleus (Bricheux et al. 2007; Gomez-Conde et al. 2000; Juliano et al. 1986) (Fig. 2a, A). The dividing nucleus also grows in size and it is initially situated between the duplicated axostyles arranged side-by-side (Fig. 2b, B–C). During metaphase, the attractaphore appears to duplicate (Marlene Benchimol 2010; Bricheux et al. 2007). A set of microtubules emerges from the attractophores and connects both attractophores to each other, forming an extranuclear spindle called the paradesmose (Petrin et al. 1998). As the paradesmose elongates it drives the division of the nucleus and the daughter cells (Juliano et al. 1986; Petrin et al. 1998) (Fig. 2a, E–G). Another set of microtubules extends from each attractophore over the outside of the nucleus and forms a mitotic spindle like-structure (Gomez-Conde et al. 2000). These microtubules connect to the kinetochores of the duplicated chromosomes residing inside the nucleus via attachment of each microtubule to the nuclear membrane (Benchimol 2004; Gomez-Conde et al. 2000; Juliano et al. 1986; Petrin et al. 1998). Thus the spindle remains extranuclear at all times. The attractophores and the anchored mitotic spindle like-structures migrate towards the opposite poles of the nucleus. Thus, at the end of metaphase, two attractophores are observed flanking the nucleus, forming the poles for nuclear division (Petrin et al. 1998).

In parallel to these processes, the axostyles start to move in opposing directions causing the dividing cell to initially display a triangle or heart-shaped morphology (Fig. 2b, C–D). The basal bodies and mastigont system continue to move in opposite directions and at metaphase end up at about 180° from each other, with the two daughter cells pointing in opposite directions but remaining connected at the cell

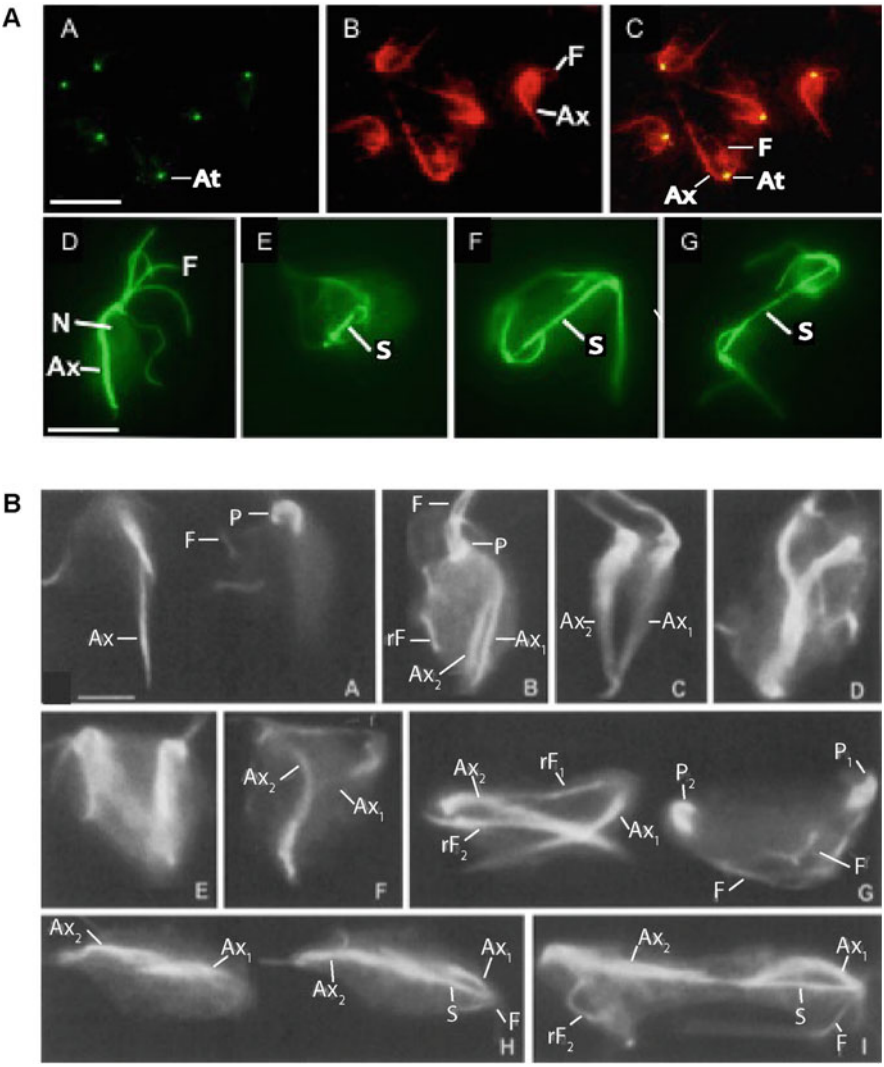

Fig. 2 Immunofluorescence of *T. vaginalis* during cell division. (**a**) Visualization of the attractophore and growing mitotic spindle during cell division. (A–C) Double immunofluorescence microscopy of extracted cytoskeletons. (A) anti-attractophore (At), green, (B) Flagella (F) and axostyle (Ax), anti-tubulin, red. (C) Attractophore co-localized near the the base of the flagella and axostyle, yellow. (E–G) A growing mitotic spindle/paradesmose (S) observed in dividing *T. vaginalis* cells. Scale bar=10 μm. (**b**) Duplication and movement of cytoskeletal structures during cell division. (A–I) anti-tubulin staining of *T. vaginalis* cytoskeletal components: axostyle (Ax), flagella (F), recurrent flagella (rF), pelta (P) and the mitotic spindle/paradesmose (S) during Interphase (A) and in dividing cells (B–I). Scale bar=3 μm. Image 3A was adapted from Copyright (2007) Elsevier. Used with permission from Bricheux et al. Identification of a new protein in the centrosome-like "atractophore" of *Trichomonas vaginalis*, Mol Biochem Parasitol. and publisher. Image 3B was adapted from Copyright (2000) Wiley. Used with permission from Ribeiro et al. Contributions of the axostyle and flagella to closed mitosis in the protists *Tritrichomonas foetus* and *Trichomonas vaginalis*, J Eukaryot Microbiol. and publisher

posterior, giving rise to an elongated cell (Benchimol 2004; Ribeiro et al. 2000) (Fig. 2b, H–I). In telophase, the nucleus is stretched and elongates, and finally separates into two daughter nuclei during the process of karyokinesis (Gomez-Conde et al. 2000). Video microscopy has revealed that flagellar beating in the opposing cells may help to complete cytokinesis, driving the physical separation of the two daughter cells which then regain their normal cell motility (Ribeiro et al. 2000). After *T. vaginalis* divides, two daughter cells are formed, each with 6 haploid chromosomes (Gomez-Conde et al. 2000).

Prior to mitosis, the Golgi in *T. vaginalis* divides through the process of Golgikinesis. The Golgi ribbon divides through medial fission cisternae by cisternae forming two smaller Golgi ribbons (Benchimol et al. 2001). When the cell divides, the Golgi ribbon in each daughter cell also travels with their associated parabasal filaments. Therefore, the two small Golgi ribbons are initially adjacent to each other side by side and then move apart and grow as the cell bodies migrate towards opposing ends. After the cell undergoes cytokinesis, a fully formed Golgi is observed in each daughter cell. Similarly, fully formed hydrogenosomes are also present in both daughter cells, in part generated through hydogenosomal fission (M. Benchimol et al. 1996; Wexler-Cohen et al. 2014).

Ultrastructural imaging has therefore uncovered a very intriguing *T. vaginalis* cytoskeletal and organellar arrangement that may have some unique and important contributions to *T. vaginalis* biology. However the specific proteins comprising these structures and organelles are less characterized. As described ahead (Table 1), large datasets from recent *omics* work are aiding in the depiction of a more comprehensive molecular characterization of these structures and organelles in *T. vaginalis*.

3.4 T. vaginalis omics*: Genome, Epigenetics, and Gene Expression*

3.4.1 The *T. vaginalis* Genome

The genome of the G3 *T. vaginalis* strain was published in 2007 as a highly fragmented assembly of sequences, thus claimed as a draft of the entire genome (Carlton et al. 2007). This ~160 Mb *T. vaginalis* draft genome is deposited in GenBank, and is also accessible at TrichDB (http://TrichDB.org), along with a variety of functional genomics data (Aurrecoechea et al. 2009). It is the largest genome among sequenced protists, containing ~60,000 predicted protein-coding genes residing in six chromosomes with many gene families highly expanded and containing many repetitive sequences (up to 65% of the genome) (Carlton et al. 2007). Since its publication, the genome has been used to reveal interesting functional properties of *T. vaginalis* and facilitated significant progress in the development of molecular biology and *omic* approaches to study the infection process. Despite the hurdles, the first *T. vaginalis* genome sequence was undoubtedly a

Table 1 Available omics resources useful for the study of *Trichomonas vaginalis*

"Omic" approach	Dataset description	Technology	Parasite strain used	References
Genomic				
Genome assembly/ annotation	Source for all gene sequence information		G3 (ATCC PRA-98)	Carlton J, *Science*. 2007
Genotype of *T. vaginalis* isolates	Identification of single nucleotide polymorphisms (SNPs) to explore population structure and genome-wide association of metronidazol genetic indicators	ddRAD-Seq	102 *T.vaginalis* clinical isolates	Bradic M, *Genome Biol Evol.* 2017
Epigenomic				
Histone Modifications Analysis	Genome wide analysis of trimethylation of histone H3 lysine 4 (H3K4me3) and Acetylation of histone H3 lysine 27 (H3K27Ac) marks	ChIP-seq using anti-H3K4me3 and H3K27Ac antibodies	T016	Song M, *Scientific Report* 2017
DNA methylation analysis	Genome wide analysis of adenine DNA methylation	MeDIP-seq using anti-6 mA antibody	B7268	Lizarraga A, *PNAS* 2020
Transcriptomic				
High-through put gene expression	Transcriptomic identification of iron-regulated and iron-independent genes	EST (expressed sequence tag) sequencing of cDNA libraries	T1	Horvathova L, *Genome Biol Evol.* 2012
High-through put gene expression	Transcriptomic analysis after *T. vaginalis* binding to fibronectin	Large-scale expressed-sequence-tag (EST) sequencing	n/a	Huang K.Y, *Infect Immun.* 2012
High-through put gene expression	Gene expression analysis during exposure to vaginal epithelial cells and oxygen stress	RNA-Seq	T016	Gould S.B, *Int J Parasitol.* 2013
High-through put gene expression	Gene expression analysis under glucose restriction	RNA-Seq	JH 31A #4 (ATCC 30236)	Huang K.Y, *Biochim Biophys Acta.* 2014

High-through put gene expression	Indentification of lncRNAs and pseudogenes	RNA-Seq	T1, T016 and FMV1	Woehle C, *BMC Genomics*. 2014
High-through put gene expression	Gene-expression analysis of cold-stress response	EST database generated from a complementary DNA library	C-1:NIH (ATCC 30001)	Fang Y.K, *J Microbiol Immunol Infect*. 2015
High-through put gene expression	Transcriptome of *T. vaginalis* in response to Tetracycline	RNA-seq	JH 31A #4 (ATCC 30236)	Huang K.Y, *Antimicrob Agents Chemother*. 2015
High-through put gene expression	Transcriptome of *T. vaginalis* to investigate the impact of iron on gene expression	RNA-seq	JH 31A #4 (ATCC 30236)	Cheng W.H, *Parasit Vectors*. 2015
High-through put gene expression	Gene expression profiles of *T. vaginalis* cultured in the presence or absence of apicidin, a histone acetylation inhibitor	RNA-Seq	T016	Song M, *Scientific Report* 2017
High-through put gene expression	Whole transcriptome analysis to survey changes in gene expression associated with Metronidazol resistance.	RNA-Seq	Nine Mz-sensitive isolates (G3, GOR69, NYCA04, NYCB20, NYCD15, NYCE32, NYCF20, NYCG31 and SD2), and three resistant isolates (NYCC3, B7268, highly resistant in vitro derivative B7268-M)	Bradic M, *Genome Biol Evol*. 2017
Proteomic[a]				
Surface proteome	Identification of surface associated proteins in strains with different adherence properties	MudPIT	Three poorly adherent strains (G3, T1, SD6) and three highly adherent strains (B7RC2, B7268, SD7)	de Miguel N, *Mol Cell Proteomics*. 2010
Hydrogenosomal proteome	Identification of hydrogenosomal proteins	LC-MS/MS	T1	Schneider R.E, *Int J Parasitol*.2011
	Proteomic analysis during *T. vaginalis* binding to fibronectin	2-dimensional gel electrophoresis	n/a	Huang K.Y, 2012

(continued)

Table 1 (continued)

"Omic" approach	Dataset description	Technology	Parasite strain used	References
Exosome proteome	Identification of proteins from released exosomes	MudPIT	B7RC2 (ATCC 50167)	Twu O, *Plos Pathogens*. 2013
Hydrogenosomal proteome	Proteomes of hydrogenosomes obtained from cells cultivated under iron-rich and iron-deficient conditions	iTRAQ-LC-MS	T1	Beltrán N.C, *PLoS One* 2013
Immunoproteome	Immunoproteome of *T. vaginalis* grown in the presence of Zn2	LC-ESI-QUAD-TOF	CNCD147 and HGMN01	Quintas-Granados L.I, *Mol Cell Proteomics*. 2013
Rhomboid 1 protease substrate identification	TvROM1 substrate identification from supernantant using quantitative proteomics	Stable isotope dimethyl labeling of cell supernatants and quantitative proteomics	RU393 (ATCC 50142)	Riestra A, *PLoS Pathog* 2015
	Proteomic Analysis of Trophozoites and Pseudocysts	LC-MS/MS	FMV1	Dias-Lopes G, *J Proteome Res.* 2018
Palmitoylproteome	Identification of palmitoylated proteins	LC-MS/MS	B7RC2 (ATCC 50167)	Nievas Y.R, *Mol Cell Proteomics.* 2018
MVs proteome	Identification of proteins from released microvesicles	LC-MS/MS	B7RC2 (ATCC 50167)	Nievas Y.R, *Cell Mol Life Sci.* 2018
Secretome	Comprehensive profiling of the *T. vaginalis* secretome	High-resolution nano LC coupled with ESI-linear-ion trap and MS/MS LF	Tv17– 48	Štáfková J, *Mol Cell Proteomics* 2018
Metabolomic				
	Comprehensive metabolomic analysis of amino acid metabolites in the hydrogenosome	LC-FT MS	JH 31A #4 (ATCC30236)	Huang K.Y, *J Microbiol Immunol Infect.* 2019

[a]Research published in the last 10 years. For previous proteomic studies see: Ryan et al. (2011a)

success, improving our understanding of the biology of this organism. However, postgenomic applications frequently require comparative genomics at the single nucleotide level and performing these analyses with the currently available highly fragmented genome is problematic. Hence, it is critical to continue efforts to improve *T. vaginalis* genome assembly and gene annotations of the reference strain. Additionally, sequencing of new *T. vaginalis* strains will provide invaluable information for future studies that will reveal knowledge about *T. vaginalis* genome architecture and structure.

In the last few years, high-through put methods for large-scale genetic comparison have been useful to confirm the existence of two genetically distinct *T. vaginalis* populations (Bradic et al. 2017). Interestingly, genomic characterization of different isolates showed that the extensive clinical variability in trichomoniasis is correlated with significant genetic diversity in the organism itself (Meade and Carlton 2013), as discussed in Sect. 2.5.

3.4.2 Epigenomics

Epigenomics analyzes epigenetic changes across the whole genome, that could lead to changes in gene activity and expression without altering DNA sequences. DNA methylation and histone modifications are examples of tightly regulated mechanisms that produce changes in gene expression, potentially leading to phenotypic changes. There is growing interest in *T. vaginalis* epigenetics due to its recently discovered role in the regulation of gene transcription and pathogenesis (Lizarraga et al. 2020; Pachano et al. 2017; Song et al. 2017). DNA methylation sequencing (MeDIP-Seq) and chromatin immunoprecipitation followed by sequencing (ChIP-Seq), enable precise genome-wide localization of epigenetic markers to decipher gene activity and chromatin states. In 2017, the first epigenome mapping was performed in *T. vaginalis* (Song et al. 2017). The authors described the genome-wide localization of two histone H3 modifications (H3K4me3 and H3K27Ac) and found that these marks are positively associated with active gene expression in both steady and dynamic transcriptional states (Song et al. 2017), providing the first direct evidence that epigenetic mechanisms play an essential role in transcriptional regulation of *T. vaginalis*. Pachano *et al*. also demonstrated that the binding of the initiator binding protein IBP39 to the metazoan-like initiator (Inr) element is strictly dependent on the acetylation state of the histones and the chromatin structure, exemplifying for the first time the mutual dependence and complex crosstalk among epigenetic players, chromatin structure, and basal gene expression machinery in *T. vaginalis* (Pachano et al. 2017).

Following these studies, DNA methylation of the parasite genome was first revealed by MeDIP-seq analysis. Genome-wide distribution of N6-methyladenine (6 mA), the main DNA methylation mark in *T. vaginalis*, reveals enrichment at intergenic regions (Lizarraga et al. 2020). Interestingly, bioinformatics analysis revealed the presence of transcriptionally active or repressive intervals flanked by 6 mA-enriched regions; with chromatin conformation capture experiments

suggesting that these 6 mA flanked regions are in close spatial proximity (Berndorff et al. 1994). Furthermore, with a combination of *omics* and molecular biology, a new role for 6 mA in modulating 3D genome architecture and gene expression has been proposed for *T. vaginalis* (Lizarraga et al. 2020).

3.4.3 Transcriptomics

The system-wide investigation of parasite gene expression is vital in understanding the coordinated set of events underlying particular stages of the parasite lifecycle, and can provide clues about which genes may be important during *T. vaginalis*: host interactions. These studies might possibly help us elect targets for new *T. vaginalis*-specific drugs. The transcriptomic data currently available for *T. vaginalis* reflects the complexity of the genome. Only about half of the encoded genes appear to be expressed (Gould et al. 2013). Interestingly, 93% of the encoded gene families are expressed together with many hundred pseudogenes and long non-coding RNAs (lncRNAs) whose expression is driven independently from neighboring genes (Woehle et al. 2014). Transcriptome comparisons have further demonstrated that individual members of gene families are differentially regulated upon environmental changes (Gould et al. 2013; Horvathova et al. 2012; Huang et al. 2014). In particular, the exposure to host cells results in differential expression of hundreds of genes within minutes (Gould et al. 2013). Additionally, changes in gene expression upon glucose restriction (Huang et al. 2014), cold-stress response (Fang et al. 2014), oxygen stress (Gould et al. 2013) or iron deficiency (Cheng et al. 2015) were observed; reflecting the rapid adjustment of the parasite to changing environments and the existence of a sophisticated mechanism that regulates adaptation. Finally, changes in gene expression associated with resistance to metronidazole (Bradic et al. 2017), the drug utilized to treat *T. vaginalis* infections (discussed in Sect. 6.1), or upon tetracycline treatment (Huang et al. 2015) have also been observed.

Analysis of the *T. vaginalis* genome and the chromatin landscape has given insight into various aspects of *T. vaginalis* biology. Employment of various *omics* approaches has further revealed how the parasite varies the expression of its large coding arsenal and how quickly it can do so. There is a great interest to further explore how the parasite globally coordinates expression of the molecular factors that facilitate host colonization, described in the next section.

4 Part 4: Host-Parasite Interactions

T. vaginalis is an extracellular pathogen of the human mucosa. As such, it must attach to host cells to establish infection. This process, termed cytoadherence, is thus crucial for its lifestyle. Often, cytoadherence is followed by killing of the host cell. However, this is not always the case. We have seen that this parasite also displays contact-independent modes of eliciting host cell death. In this section, we will

describe the process of parasite cytoadherence and the involvement of specific molecular mediators. We will depict how this parasite promotes the killing of host cells by cytolytic factors or by modulating known pathways that induce host cell death. The underlying mechanisms of virulence that might be contact-dependent or -independent, including recent descriptions of extracellular vesicles produced by this parasite, will also be discussed here. Finally, looking at the other side of the equation, we will examine the involvement of host immune responses—including effector cells and molecules of the innate and adaptive immune response—in helping clear the infection and playing decisive roles on immunopathogenesis of trichomoniasis. As we move closer toward understading host-parasite interactions, this knowledge will be of therapeutic interest to block specific players to stymie parasite colonization, prevent ensuent tissue damage, and/or help boost a protective immune response against this parasitic infection.

4.1 T. vaginalis *Cytoadherence and Host Cell Killing*

4.1.1 Morphology and Dynamics of *T. vaginalis* in Contact with Host Cells

T. vaginalis binds to a myriad of epithelial cells of the female and male reproductive tract as well as spermatozoa, red blood cells, and leukocytes (Benchimol et al. 2008; Fiori et al. 1997; Mercer and Johnson 2018; Rendon-Maldonado et al. 1998; Rosset et al. 2002; Vazquez-Carrillo et al. 2011). Microscopy studies have characterized the morphology and dynamics of *T. vaginalis* adhesion to host cells. Immediately or within 5 min after co-incubation, *T. vaginalis* can be observed tightly bound to human epithelial cells (Arroyo et al. 1993; Furtado and Benchimol 1998; Rasmussen et al. 1986) changing its tear drop/ovoid shape to a flat, ameboid form as shown in Fig. 3 (Arroyo et al. 1993; Furtado and Benchimol 1998; Midlej and Benchimol 2010; Rasmussen et al. 1986). Arroyo et al. described this morphological change upon contact with the host cell, wherein the parasite body elongates, pseudopodia are formed, and the parasite continues to flatten out on top of the host cell gaining a "fried-egg" appearance (Arroyo et al. 1993). In scanning electron micrographs, tight apposition between the parasite and the host cell plasma membranes can be observed throughout the cell body of the parasite, potentially to maximize adherence with the host cell (Arroyo et al. 1993; Furtado and Benchimol 1998; Rasmussen et al. 1986). In addition, cytoplasmic projections protrude from the parasite and interdigitate with microvilli of vaginal epithelial cells (Arroyo et al. 1993; Furtado and Benchimol 1998). Intriguingly, transmission electron microscopy has revealed areas of continuity between the parasite and the host cell plasma membranes (Furtado and Benchimol 1998). Midlej and Benchimol also imaged *T. vaginalis* in the process of "pinching-off" or pulling up microvilli of bovine oviduct epithelial cells and endocytosing microvilli (Midlej and Benchimol 2010). These events resemble trogocytosis, a process by which cellular nibbling by the gut extracellular

Fig. 3 Scanning electron microscopy of *T. vaginalis* trophozoites under ovoid and amoeboid forms. (**left**) *T. vaginalis* (green), with flagella (blue), recurrent flagella (orange) and axostyle (green protruding from the cell posterior) in its ovoid form. (**right**) Amoeboid *T. vaginalis* (green) adhered to a vaginal epithelial cell (pink). Images show significant morphological changes that occurs during host-cell adhesion. Image was provided as a gift by Dr. Antonio Pereira-Neves, Oswaldo Cruz Foundation-Fiocruz, Aggeu Magalhães Institute (Pernambuco, Recife, Brazil)

protozoan *Entamoeba histolytica* leads to membrane exchange and contributes to host-cell lysis (Ralston 2015b). However, whether *T. vaginalis* uses trogoycytosis to kill host epithelial cells remains to be formally tested.

T. vaginalis motility also contributes to the nature of host-cell interactions. Videomicroscopy of *T. vaginalis* reveals that parasites can remain attached long-term to host cells or display dynamic attachment: attaching and then detaching and moving on to other host cells (Furtado and Benchimol 1998). Thus, it has been proposed that *T. vaginalis* can display a "hit-and-run" effect (Furtado and Benchimol 1998). However, the dynamics of *T. vaginalis* interactions with host cells remains to be quantified and more fully characterized with different host cell types.

T. vaginalis can also adhere to host cells in aggregates, also referred to as clumps or microcolonies. Aggregate adherence to host cells occurs rapidly (Arroyo et al. 1993; Rasmussen et al. 1986), and clumps attached to host cells remain visible after several hours (Midlej and Benchimol 2010; Rasmussen et al. 1986). Parasite aggregate adherence appears to be a more common phenotype of fresh clinical isolates and more virulent strains of *T. vaginalis* (Coceres et al. 2015; Midlej and Benchimol 2010), indicating that clumping could be an important mechanism of enhanced pathogenesis. Interestingly, parasite-parasite membrane interdigitations amongst aggregates attached to host cells are also visible by scanning electron microscopy, indicating a very strong association between individual parasites within clumps (Arroyo et al. 1993). Recent studies have started to identify the *T. vaginalis*

membrane proteins that help mediate these parasite-parasite interactions. Overexpression of the tetraspanin protein 8 (TvTSP8, TVAG_008950) and a cadherin-like protein (TvCLP, TVAG_393390) led to increased parasite clump formation (defined as an aggregate of $\sim$ 10 or more parasites in both studies) (YP Chen et al. 2019; Coceres et al. 2015; Nievas et al. 2018b) indicating that these proteins could be virulence factors aiding in clumping. We are beginning to learn how specifc features of these surface proteins help to coordinate their involvement in the clumping process. For example, calcium ions are required for maximal aggregate formation mediated by CLP, possibly due to conformational stabilization of CLP similarly to calcium's effects on mammalian cadherin proteins (YP Chen et al. 2019). The C-terminal tail of TvTSP8 has been found to be essential to target TvTSP8 to the parasite cell surface and the C-terminal tail may also be important to engage with intracellular cytoskeletal and signaling proteins (Coceres et al. 2015). Additionally, palmitoylation of TvTSP8 may also be necessary to promote parasite clumping, as inhibition of S-acylation led to reduced clump formation by TSP8 (Nievas et al. 2018b). Interestingly, TvTSP8 and TvCLP are both upregulated upon binding to ectocervical cells, indicating that clumping may be increasingly induced upon contact with host cells, although this remains to be formally investigated. Collectively, this indicates that *T. vaginalis* clumping behavior can be regulated.

4.1.2 Mechanisms of Cytoadherence-Dependent and Independent Host Cell Killing

Generally, after *T. vaginalis* attaches to host cells, lysis of host cells ensues (Lustig et al. 2013). Host cell damage inflicted by *T. vaginalis* has been observed via various experimental findings. For example, co-incubation of host cells with parasites can lead to visible gaps in cell monolayers as shown in Fig. 5b–c (Furtado and Benchimol 1998; Lin et al. 2015; Lustig et al. 2013; Ma et al. 2011; Puente-Rivera et al. 2017). Measurements of decreased transepithelial electrical resistance, indicative of reduced monolayer integrity following *T. vaginalis* infection has also been observed (Guenthner et al. 2005). Lastly, co-incubation of fetal membranes with *T. vaginalis* also causes tissue weakening (Draper et al. 1995)**.** Therefore in vivo, *T. vaginalis* cytoadherence may compromize the integrity of epithelial barrier function in infected tissues. The dynamics of host cell destruction also vary by *T. vaginalis* strain, host cell type, and the ratio of parasites to host cells (Mercer and Johnson 2018), findings that together may account for the varied nature of symptomology in humans after *T. vaginalis* infection.

As described above, after parasite attachment to host cells, the parasite can ingest fragments of host epithelial cells. One study found that a large majority of epithelial cells had signs of necrosis, including plasma membrane rupture and release of cytosolic content, following adherence of *T. vaginalis*. Researchers also found that host cell organelles were ingested by the parasite (Midlej and Benchimol 2010). However, membrane blebbing and condensed chromatin, indicative of apoptosis, have also been observed following *T. vaginalis* interaction with host cells (Midlej

and Benchimol 2010). A recent study by Lin and collegues reported that after incubation of a cervical epithelial cell line with *T. vaginalis*, 70% of cells displayed "signs of disruption", while other cells displayed a morphology indicative of necrosis or apoptosis (Lin et al. 2015), indicating that different forms of cell death can be infilicted in a population of host cells after contact with *T. vaginalis*. It is also possible that *T. vaginalis* "feeds off" ingested host cell material, as host cell debris has been found inside *T. vaginalis* vacuoles, that are subsequently trafficked to the lysosomes (Midlej and Benchimol 2010). The specific molecular interactions and events that occur during parasite adherence to host cells and how they trigger host cell death remain largely unknown and may vary by host cell type. Therefore, we will present knowledge about cytoadherence-dependent cytolysis in general and then examine findings about host cell death by cell types.

Parasite attachment to host cells is detected quickly, and highly virulent strains can lyse host cells soon after (Alderete and Garza 1985; Lustig et al. 2013; Okumura et al. 2008). In the most comprehensive comparison of *T. vaginalis* strains, Lustig et al. compared the attachment and lytic properties of 26 *T. vaginalis* strains co-incubated with the ectocervical cell line (Ect-1) or the benign prostate hyperplasia cell line (BPH-1). A significant correlation between the parasite's adhesive and cytolytic properties was found among the weakly cytolytic *T. vaginalis* stains, whereas this correlation was not significant in the strongly cytolytic strains, indicating that a threshold of cytoadherence may need to be reached, after which host-cell killing proceeds. In addition, separating parasite and host cells using transwell inserts that prevented physical contact blocked lysis of all 26 *T. vaginalis* strains tested (Lustig et al. 2013), showing that killing of epithelial cells is contact-dependent. Increasing the *T. vaginalis* multiplicity of infection in cytolysis assays also leads to increasing amounts of host cell lysis (Alderete and Pearlman 1984; Gilbert et al. 2000). Thus, increasing the amount of parasites that bind to and physically damage host cells potentially augments the lysis of host cells. However, future single-cell tracking analysis is necessary to discern how parasite contact and adherence to host cells is mechanistically linked to the induction of host cell death.

Inhibition of several *T. vaginalis* cellular processes has shed light on factors that contribute to *T. vaginalis* cytoadherence and host-cell killing. Initial studies involving *T. vaginalis* treatment with cyclohexamide and proteases revealed an inhibition on host cell binding, indicating that active protein synthesis by *T. vaginalis* and surface-localized proteins mediate parasite attachment to host cells (Alderete and Garza 1985). As mentioned in Sect. 3.4.3, recent transcriptomic analysis of *T. vaginalis* uncovered that exposure to host cells induces differential expression of hundreds of genes within minutes, identifying genes that may contribute to *T. vaginalis* interaction with host cells (Gould et al. 2013). Upregulation of several proteins with potential roles in pathogenesis have also been found following host cell encounter (YP Chen et al. 2019; Coceres et al. 2015; de Miguel et al. 2010; de Miguel et al. 2012; Gould et al. 2013). Additionally, post-translational modifications may also regulate the activity of these *T. vaginalis* adhesins, as dephosphorylation by the phosphatase TvPP1γ and protein palmitoylation also affect parasite attachment (Munoz et al. 2012; Nievas et al. 2018b). Therefore, *T. vaginalis* likely employs a

speedy and multi-faceted transcriptionally and post-translationally-regulated response to maximize colonization.

To date, the only *T. vaginalis* ligand and host receptor pair fully characterized to participate in attachment is *T. vaginalis* surface lipoglycan (TvLG) binding to the host cell galectin-1 protein (Bastida-Corcuera et al. 2005; Fichorova et al. 2016; Okumura et al. 2008; Ryan et al. 2011b; Singh et al. 1994). *T. vaginalis* contains ~3 × 10^6 TvLG molecules that form a dense glycocalyx on the parasite surface (Singh et al. 1994). Chemically-mutagenized parasites that have altered TvLG composition display reduced adherence and lysis of ectocervical cells (Bastida-Corcuera et al. 2005). Similarly, knockdown of galectin-1 on host cells caused a 20% decrease in parasite attachment (Okumura et al. 2008). Galectin-1 is a homodimer with two carbohydrate recognition domains, and is hypothesized to act as a bridge binding *T. vaginalis* TvLG on one end, and galactose on the host cell on the other end (Ryan et al. 2011a). Specifically, the TvLG glycoconjugate's outermost N-acetyllactosamine (LacNAc) and lacto-N-biose (LNB) side chains help mediate the parasite binding to galectin-1 on host ectocervical cells (Ryan et al. 2011a).

Two additional potential adherence ligands are TVAG_140850, and TVAG_240680, which are upregulated upon host-cell encounter, and whose overexpression contributed to enhanced attachment to host cells (Gould et al. 2013; Handrich et al. 2019). Interestingly, these proteins are members of the Pmp and BspA families, respectively. These gene famlilies are highly expanded in the *T. vaginalis* genome, predicting a functional importance (Carlton et al. 2007; Hirt et al. 2011). Furthermore, exogenous expression of TVAG_140850 and TVAG_240680 in a bird-infecting *Tetratrichomonas gallinarum*, also increased ability of this trichomonad to bind vaginal epithelial cells (Handrich et al. 2019). However, the host cell receptor for these adhesins remains unknown.

T. vaginalis proteases also clearly play a role in cytoadherence and killing. It is estimated that the *T. vaginalis* genome encodes hundreds of peptidates, with one study identifying 310, and another reporting 447 (Carlton et al. 2007; Hirt et al. 2011). Thus *T. vaginalis* has one of the largest predicted degradomes amongst eukaryotic protists. The use of protease inhibitors that target cysteine, serine, and metalloproteases has helped to reveal the involvement of proteases in *T. vaginalis*: host interactions. Protease inhibitors reduce the ability of *T. vaginalis* to attach to and/or lyse host cells (Alvarez-Sanchez et al. 2000; Arroyo and Alderete 1989; De Jesus et al. 2009; Hernandez-Gutierrez et al. 2004; Ma et al. 2011; Mendoza-Lopez et al. 2000; Puente-Rivera et al. 2017; Quan et al. 2014; Rendon-Gandarilla et al. 2013; Riestra et al. 2015; Sommer et al. 2005). Additionally, inhibiting protease function with blocking antibodies also decreases parasite attachment and/or host cell lysis (Alvarez-Sanchez et al. 2000; Cardenas-Guerra et al. 2013; Hernandez et al. 2004; Mendoza-Lopez et al. 2000; Miranda-Ozuna et al. 2019; Puente-Rivera et al. 2017; Rendon-Gandarilla et al. 2013; Rivera-Rivas et al. 2020). Furthermore, *T. vaginalis* protease expression and activity levels differ under varying levels of Fe^{2+}, Zn^{2+}, polyamines, oxygen, glucose, and between *T. vaginalis* strains with different virulence properties (Alvarez-Sanchez et al. 2008, 2007; Arroyo et al.

2015; Cuervo et al. 2008; De Jesus et al. 2009; Garcia et al. 2005; Gould et al. 2013; Hernandez-Romano et al. 2010; Kummer et al. 2008; Miranda-Ozuna et al. 2019; Puente-Rivera et al. 2017; Quintas-Granados et al. 2013; Rivera-Rivas et al. 2020). Therefore, proteases likely mediate and modulate *T. vaginalis* cytoadherence and host-cell killing, and certain proteases may mediate particular roles in different microenvironments within the host.

Specifically, cysteine proteases constitute the largest mechanistic and most characterized class of proteases in *T. vaginalis* (Carlton et al. 2007; Hirt et al. 2011). *T. vaginalis* cysteine proteases localize to lysosomes, the Golgi complex, cytosolic vacuoles, vesicles, the parasite cell surface, and can also be secreted (Hernandez-Gutierrez et al. 2004; Mendoza-Lopez et al. 2000; Rendon-Gandarilla et al. 2013; Riestra et al. 2015). Cysteine proteases are known to be active during natural infection, because vaginal lavages of infected women contain the active proteases, as well as antibodies raised against them. While cleavage of extracellular matrix proteins by some of these cysteine proteases has been reported (Alvarez-Sanchez et al. 2000; Hernandez-Gutierrez et al. 2004; Rendon-Gandarilla et al. 2013), how inhibition of *T. vaginalis* proteases leads to the observed effects on modulating *T. vaginalis* adherence to host cells and host cell lysis remains largely unknown. The identification of the parasite and/or host substrates during these processes will shed light on the mechanisms of action at play.

Gain of function studies utilizing recent advances in *T. vaginalis* molecular biology coupled with bioinformatics or proteomics have also allowed the discovery of additional *T. vaginalis* surface proteins and parasite factors involved in cytoadherence and killing. De Miguel and colleagues conducted a quantitative proteomics study that identified and compared the *T. vaginalis* surface proteins in highly adherent strains and weakly adherent strains (de Miguel et al. 2010). The study revealed 11 proteins that were more abundant in the highly adherent *T. vaginalis* strains. Overexpression of two of these proteins, TvBAP1 (TVAG_166850) and TvBAP2 (TVAG_244130), led to a >2 fold increase in attachment to the ectocervical cells. The putative intramembrane protease TvROM1 was also identified in the *T. vaginalis* cell surface proteome, and membrane localization was confirmed in a later study (Riestra et al. 2015). Exogenous expression of TvROM1 led to a 1.6 fold increase in parasite attachment and a 4.2 fold increase in lysis of ectocervical cells compared to empty vector control transfectants (Riestra et al. 2015). Riestra and colleagues also identified two substrates, TvBAP1 and TVAG_280090, cell surface proteins functionally annotated as hypothetical proteins. Mutation of the TvROM1 cleavage site in TvBAP1 led to increased TvBAP1 surface amounts. These findings suggest that *T. vaginalis* may modulate the presence of certain cell surface adhesins during parasite attachment and host cell lysis. It remains to be determined whether the observed effects on host cell attachment and host cell lysis are mediated by cleavage of the same or different parasite and/or cell surface proteins.

Structure predictions have helped to reveal additional *T. vaginalis* proteins involved at the parasite: host interface. Bioinformatic analysis of two surface proteins implicated in adherence, TvBAP1 and TVAG_280090, predicted cadherin-like

tertiary structures (Riestra et al. 2015). In multicellular organisms, cadherin-like proteins mediate cell-cell interactions and cell-cell adhesion (Abedin and King 2010). Further searches for cadherin-like proteins in *T. vaginalis*, led to the functional characterization of TVAG_393390 (TvCLP). Exogenous expression of TvCLP led to a 3.5 fold increase in *T. vaginalis* adherence to the ectocervical cells and a 3.3 fold increase in *T. vaginalis* lysis of these cells, compared to empty vector transfectants (Chen et al. 2019). Calcium binding was also found to be necessary for TvCLP's role in promoting host cell attachment and cytolysis. Investigating the evolutionary history of the cadherin-like proteins and possible homologs in other parasitic protozoans is of interest, as these may be evolutionary relics of mammalian cadherin-like proteins and have conserved functional roles in pathogenesis.

T. vaginalis may also utilize its putative large arsenal of virulence factors to respond differently in the female vs. male host. For example, differences in iron availability occur during female menses and several studies have revealed that iron can modulate the expression of various cysteine proteases (Arroyo et al. 2015; Figueroa-Angulo et al. 2012). In the male, prostate zinc levels are the highest compared to other organs (Cui et al. 2015). Quintas-Granado and colleagues identified and characterized a *T. vaginalis* metalloprotease, TvMP50 (TVAG_403460), that was recognized by sera collected from a *T. vaginalis*-infected male but was not detected by sera from a *T. vaginalis*-infected female or uninfected male and female controls, indicating that this metalloprotease is only expressed during infection in men. TvMP50 expression and proteolytic activity was also upregulated in the presence of increased Zn^{2+} concentrations. TvMP50 also caused killing of a prostate epithelial cell line (Puente-Rivera et al. 2017). Interestingly, an antibody raised against TvMP50, reduced the cytolytic activity of TvMP50 and the inhibitory effects were more pronounced under higher Zn^{2+} concentrations. Together, these studies highlight that Zn^{2+} concentrations may regulate the cytolytic activity of TvMP50 and that this metalloprotease could play an important and differential role in male trichomoniasis.

Lastly, the secretion of extracellular vesicles by *T. vaginalis* (described in Sect. 4.2) also helps to bolster parasite attachment to host cells. Exosomes isolated from highly adherent strains can increase the adherence of the weakly adherent G3 strain to ectocervical cells (Twu et al. 2013). Furthermore, *T. vaginalis* exosomes from two strains that display higher adherence to a prostate cell line compared to an ectocervical cell line (Lustig et al. 2013) similarly confer this preferential binding ability to the G3 strain. Therefore, exosomes may prime host cells for maximal *T. vaginalis* attachement and for successful colonization of the two different environments encountered within the male and female hosts.

4.1.3 A Closer Examination of Host Cell Death Induced by *T. vaginalis*

As described in Sect. 4.1.2, signatures of both host-cell necrosis and apoptosis have been detected by different groups of researchers following exposure to *T. vaginalis*. However, recent advances in the field of cell death have identified several additional

cell death pathways, and revealed extensive crosstalk between different cellular death pathways (Galluzzi et al. 2018; Rogers and Alnemri 2019; Rogers et al. 2019; Yu et al. 2014), adding some nuance to the necrosis vs. apoptosis dichotomy. It is therefore likely that host cells may die in context and cell-type specific ways following *T. vaginalis* encounter, as we outline below, and summarize in Fig. 4. Furthermore, it may be necessary to build on the knowledge generated from the existing studies and perform future analysis of host cells *en route* to dying after contact with *T. vaginalis*, to better define how *T. vaginalis* induces cell death. Below, we discuss the evidence supporting each cell-death pathway in contexts where it was observed.

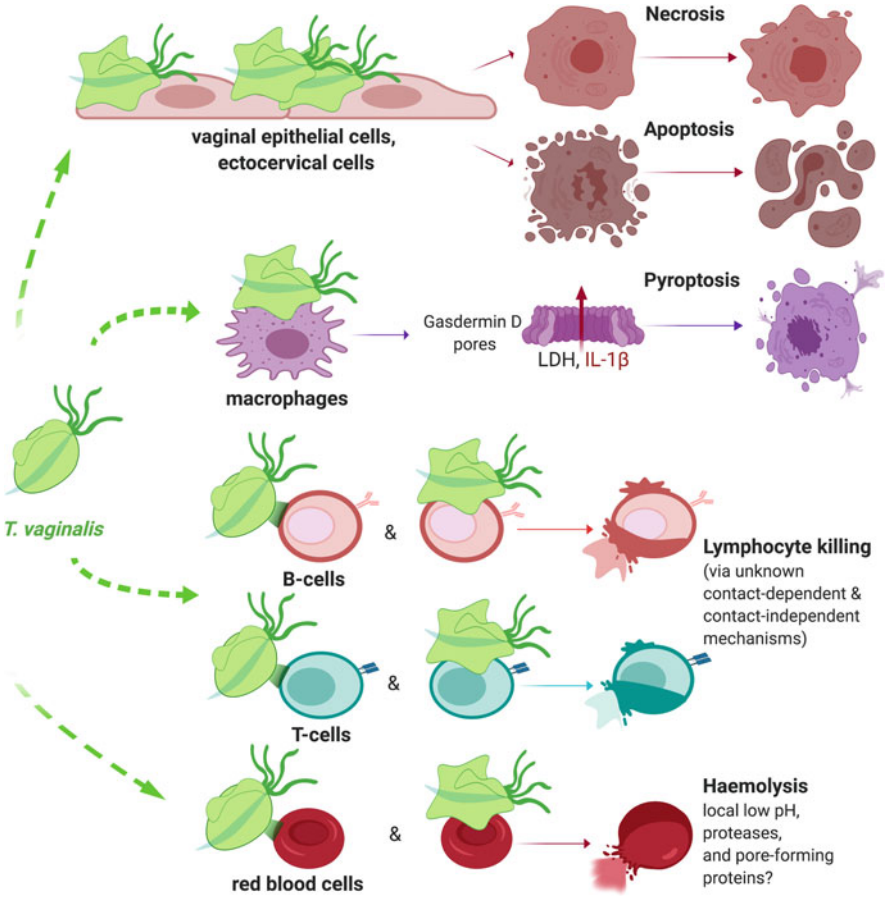

Fig. 4 *T. vaginalis* cytoadherence and lysis of various host cell types. Schematic of the various cell types to which *T. vaginalis* attaches and kills via the various cell death pathways shown. *T. vaginalis* can also kill B-cells, T-cells, and red blood cells via contact-independent cytolysis. Figure created with BioRender.com

4.1.3.1 Host Cell Apoptosis

Researchers have found that DNA fragmentation, a key signature of apoptosis, occured in two cervicovaginal cell types following culture with *T. vaginalis* (Quan et al. 2014; Sommer et al. 2005). In fact, in the absence of whole-live parasites, secreted products from the parasites showed a similar effect (Sommer et al. 2005), indicating that cytoadherence-independent cell death could proceed via apoptosis during infection. Quan and colleagues also detected many other signs of apoptosis such as cleavage of the initiator caspase-9, the executioner caspase-3, the caspase-3 substrate PARP, and increased cytolosolic cytochrome c , all signatures of intrinsic cellular apoptosis (Rogers and Alnemri 2019). However, since detection of these and other markers of apoptosis largely relies on assessing cleavage products, *T. vaginalis'* large degradome activity may complicate discernment of cell death pathways. Single cell analysis of dying host cells after encounter with *T. vaginalis* and secreted products is therefore necessary in order to fully understand the order of signaling events that induce host cell killing.

How *T. vaginalis* encounter with host cells could physically lead to the activation of intrinsic apoptosis also remains largely unknown. In the studies described above, Quan et al. identified that *T. vaginalis* metalloproteases contribute to activate apoptosis (Quan et al. 2014). A later study by Quan and colleagues identified that upon co-incubation of a cervical cell line with *T. vaginalis*, host cells increased production of intracellular and mitochondrial reactive oxygen species (ROS) production, and that inhibition of ROS production during infection with *T. vaginalis* caused reduced levels of apoptosis markers (Quan et al. 2017). Therefore, upon *T. vaginalis* infection of cervical epithelial cells, a *T. vaginalis*-generated signal as well as increased oxidative stress in the host cells could contribute to apoptosis activation.

4.1.3.2 Host Cell Pyroptosis

Pyroptosis, or "a fiery death," is a newly discovered form of cell death that is commonly activated as part of the innate immune response to pathogens and host cell injury, and results in strong inflammatory signals being sent from the dying cell (Galluzzi et al. 2018). Pyroptosis starts when Pattern-Recognition Receptors (PRRs) in the cytosol form multi-protein complexes called inflammasomes. Inflammasome assembly in turn leads to the activation of inflammatory caspases which can cleave the gasdermin D protein (Kovacs and Miao 2017; Shi et al. 2015). Importantly, the gasdermin D N-terminal cleavage fragment can then insert itself in the plasma membrane, oligomerize, and form pores (Ding et al. 2016), thus causing cell death (Kovacs and Miao 2017; Liu et al. 2016; Shi et al. 2015). The overall cellular features of pyroptosis are chromatin condensation, cellular swelling, and fast cell lysis (Galluzzi et al. 2018). Additionally, pyroptosis is almost always accompanied with secretion of the pro-inflammatory cytokines IL-1β and IL-18 (Galluzzi et al. 2018; He et al. 2015; Shi et al. 2015).

As discussed in Sect. 4.3.2, two separate research groups found that *T. vaginalis* induces inflammasome activation in a prostate epithelial, and macrophage cell line, respectively (Gu et al. 2016; Riestra et al. 2019). Both groups detected an increase in processing of the pyroptosis-specific caspase-1. During pyroptosis, the gasdermin D pores that assemble in the host cell membrane are 10–14 nm in diameter and thus proteins that are smaller than the pore size, such as LDH (8-10 nm), can exit the cell (Ding et al. 2016; He et al. 2015). Riestra and colleagues found that inflammasome and caspase-1 inhibition caused a reduction in LDH release after co-incubation of THP-1 macrophages with *T. vaginalis*. Furthermore, *T. vaginalis* also had a reduced ability to lyse gasdermin D knockout cells compared to control cells, further supporting pyroptosis as the mechanism of macrophage host-cell killing. The authors of these studies thus hypothesized that *T. vaginalis* induces pyroptosis in host cells. Further investigation at a morphological level is warranted to more fully characterize the induction of pyroptosis after contact with *T. vaginalis* and to investigate the effects of inflammasome activation on the host.

Recently, it has also been identified that inflammasome activation can lead to apoptosis, because the N-terminal gasdermin D cleavage fragment can also form pores in the mitochondrial membrane, contributing to mitochondrial outer membrane permeabilization and the classic downstream apoptotic events such as cytochrome c release (Rogers and Alnemri 2019; Rogers et al. 2019; Yu et al. 2014). Chang et al. detected increased Annexin V staining, cytochrome c release into the cytosol, and apoptotic caspase activity after co-incubation of a macrophage cell line with *T. vaginalis* (Chang et al. 2004), all indicators of apoptosis. However, nucleosomal degradation and Annexin V^+ staining are also detected during pyroptosis, as gasdermin D pores allow Annexin V access to the inner leaflet of the plasma membrane (Abe and Morrell 2016; Man and Kanneganti 2016). Therefore, it remains to be determined, whether induction of apoptosis downstream of pyroptosis may reconcile signs of pyroptosis versus apoptosis observed in macrophage cell lines exposed to *T. vaginalis*, or whether each type of cell death is induced depending on differential conditions or parasite strains.

4.1.3.3 Haemolysis

T. vaginalis also lyses red blood cells (RBCs) and its hemolytic activity has been predicted to provide the parasite with a source of iron and lipids (Fiori et al. 1996), however the precise molecular interactions that drive RBC lysis also remain to be identified. Using scanning electron microscopy, Fiori and colleagues as well as Rosset and colleagues reported that tight contact is observed when *T. vaginalis* interacts with RBCs (Fiori et al. 1997; Rosset et al. 2002). Studies have also highlighted the potential involvement of both contact-dependent and contact-independent haemolysis (Fiori et al. 1993, 1996, 1997; Pindak et al. 1993; Rosset et al. 2002). However, the experimental conditions of how *T. vaginalis* secretions were collected was critical for detection of contact-independent haemolysis, as this experimental variable affected the final pH of the *T. vaginalis* supernatants, and a

more acidic pH was found to maximize haemolytic activity (Fiori et al. 1996; Pindak et al. 1993). Researchers have also found that osmoprotectants can reduce or completely inhibit the lysis of RBCs by *T. vaginalis* (Fiori et al. 1993, 1996). Thus it has been hypothesized that haemolysis may be driven by *T. vaginalis* pore-forming proteins that insert into the RBC membrane, and that a low pH that is locally induced by *T. vaginalis* adherence is needed in order to trigger this haemolytic activity (Fiori et al. 1993, 1996). Analysis of the *T. vaginalis* genome by Hirt and colleagues has revealed the presence of 12 genes coding for putative proteins belonging to the saposin-like protein family (TvSAPLIP), which display homology to pore-forming proteins of the pathogenic protists *Entamoeba histolytica* and *Naegleria fowlerii*. Some TvSAPLIPs have been detected in *T. vaginalis* secretions (Riestra et al. 2015; Štáfková et al. 2018), thus TvSAPLIP family members may participate in cell lysis including haemolysis (Hirt et al. 2011).

The weakening of the membrane-associated cytoskeleton of RBCs appears to be a key event in *T. vaginalis*-induced heamolysis. Fiori and colleagues showed that a non-secreted protease of *T. vaginalis* specifically targets and degrades the RBCs spectrin, the main component of membrane cytoskeleton: this step takes place before haemolysis occurs (Fiori et al. 1997). Recently, Cardenas-Guerra and colleagues identified that the *T. vaginalis* cysteine protease TvCP4 may also help to mediate haemolysis, as pre-incubation of the parasite with an anti-CP4 antibody or with a cysteine protease inhibitor caused a reduction in RBC lysis by *T. vaginalis* (Cardenas-Guerra et al. 2013). Future studies will help to reveal whether shared features exist between how *T. vaginalis* lyses RBCs compared to other host cell types.

4.1.3.4 Lymphocyte Lysis

Similar to the lysis of RBCs, researchers have also detected contact-dependent and contact-independent lysis of B-cells and T-cells (Mercer et al. 2016), as discussed in Sect. 4.3.5. In these studies, a highly adherent clinical isolate could lyse about 30% of T cells and about 70% of B-cells during a 4 h co-incubation, whereas the G3 lab-adapted *T. vaginalis* strain caused less B-cell lysis and no T-cell lysis. Therefore, *T. vaginalis* appears to have a preference for lysing B-cells. Furthermore, when physical contact between the parasite and lymphocytes was prevented via insertion of a membrane filter, cell lysis of both cell lines was reduced by two-fold, indicating that contact-independent factors also contribute to the killing of B- and T-cells by *T. vaginalis*. To date, no specific *T. vaginalis* factors that mediate the initial contact or downstream lysis of lymphocytes have been identified.

A plethora of molecular determinants that facilitate contact with host cells and killing of host cells have been identified. Future investigations now rely on integrating the various processes and players that have been highlighted to contribute to pathogenesis, and dissect how and when they are specifically used to mediate host cell killing and how exactly that cell death ensues. Extracellular vesicles, host commensals, *T. vaginalis'* symbionts, and the host immune system are additional

layers to be considered at the host-pathogen interface and likely to contribute to parasite pathogenesis as well, as we discuss below.

4.2 T. vaginalis *Extracellular Vesicles*

Exciting work in the past decade has revealed that *T. vaginalis* secretes Extracellular Vesicles (EVs), that the parasite putatively uses for communication among different trichomonads and with host cells, during infection (Nievas et al. 2018a; Twu et al. 2013). For a discussion of how EVs affect *T. vaginalis* cytoadherence please see Sect. 4.1, and for a discussion of how they have immunomodulatory roles see Sect. 4.3. Here, we will discuss their identification, biogenesis, contents, and mechanism of delivery to host cells.

EVs are a membrane-bound secretome product that contribute to the adaptive capabilities of microbial cells through delivery of proteins, lipids and nucleic acids to both adjacent and distant cells. The international society of extracellular vesicles (ISEV) endorses "extracellular vesicles" as the generic term for particles naturally released from the cell that are surrounded by a lipid bilayer and cannot replicate (Thery et al. 2018). Depending on their size and mechanisms of biogenesis, EVs are classified into three major categories: exosomes, microvesicles, and apoptotic bodies. Exosomes range in size from 30 to 150 nm in diameter, and are formed by the fusion of intracellular multivesicular bodies with the plasma membrane. Ectosomes, or microvesicles (MVs), are 100–1000 nm in diameter, and are shed directly from the plasma membrane (van Niel et al. 2018). Apoptotic bodies are larger than 1000 nm in size and released during apoptotic cell death (Caruso and Poon 2018). Despite apparent differences in the mechanism of formation, it is experimentally difficult to discriminate among these vesicle populations after they are secreted from cells, due to the overlap in their sizes. However, as researchers identified more vesicular proteins, it became apparent that EVs contain a specific subpopulation of proteins rather than a random pool of molecules from their cells of origin (Choi et al. 2015), indicating that it should be feasible to define a panel of markers for different kinds of EVs. Despite the enrichment of EV contents, there is currently no clearly descriptive physical property or molecular marker that can unambiguously distinguish exosomes from MVs (Choi et al. 2015). Hence, assigning specific functions to the different EVs populations remains a challenge.

Nonetheless, the analysis of *T. vaginalis* EVs has become an active endeavor in the study of pathogenesis for this parasite, and so far, the release of both exosomes and MVs has been documented (Nievas et al. 2018a; Olmos-Ortiz et al. 2017; Twu et al. 2013). Twu and colleagues isolated vesicles smaller than 200 nm (with a mean diameter of 95 nm) mainly enriched in exosomes, and Nievas and colleagues designed a protocol to isolate vesicles from the same highly adherent strain (B7RC2) ranging between 200 and 800 nm (with a mean diameter of 380 nm) enriched mainly in MVs (Nievas et al. 2018a), indicating that the same strain of *T. vaginalis* can secrete both exosomes and MVs. Interestingly, the secretion of both

types of vesicles increases upon exposure of the parasite to host cells, suggesting that EVs play an important role in pathogenesis (Nievas et al. 2018a; Twu et al. 2013). To begin to make predictions about their function, researchers performed proteomic analysis of these vesicles, and identified 215 and 592 proteins in exosomes and MVs proteomes, respectively. Interestingly, there was a good deal of overlap, as 167 out of 215 exosome proteins were also found in the MVs proteomic survey, similar overlap has also been observed in other studies (Abels and Breakefield 2016). However, it is important to consider that a minimum level of contamination is inevitably present in the samples due to vesicle aggregation. Nevertheless, as 425 proteins were unique to MV's, this could indicate that these vesicles may be selectively loaded with specific groups of proteins that exert specific functions. Many of the molecules identified in both proteomes are commonly found in EVs proteomes from other organisms. These include members of the vesicle trafficking-related complex such as ESCRT factors (VPS32 and CHMP1) (Iriarte et al. 2018; Schmidt and Teis 2012); Rab GTP-ases and SNARE-complex proteins (VAMP, syntaxin, Vti1) (Fader et al. 2009; Ostrowski et al. 2010); cytoskeletal proteins (actin, tubulin, villin, plastin) (Mathieu et al. 2019); metabolic enzymes (enolases, malic enzyme, glyceraldehyde 3-phosphate dehydrogenase); ribosomal proteins and tetraspanins (TvTSP1 in exosomes (Twu et al. 2013) and TvTSP8 in MVs (Nievas et al. 2018a).

Tetraspanin proteins (CD9, CD63, and CD81) are often used as markers of EVs in mammalian cells (Kowal et al. 2016; Mathivanan et al. 2010). Tetraspanins are transmembrane proteins that organize specialized membrane domains termed tetraspanin-enriched microdomains (TEMs) which may play a role in EVs biogenesis, cargo selection and/or binding and uptake by target cells (Andreu and Yanez-Mo 2014). Considering the presence of the two tetraspanins, TvTSP1 and TvTSP8 in the exosome and MVs proteome, respectively (Nievas et al. 2018a; Twu et al. 2013), these proteins could potentially be used as markers of EVs in *T. vaginalis.* However, it is unlikely that TSP proteins will be useful as general markers of all protozoan EVs, as they are present in some, but not all protozoan genomes. Specifically, TSP-like sequences have been found in *E. histolytica*, *Leishmania* spp., *Trypanosoma cruzi,* and *Trypanosoma brucei*, but no TSP-like sequences were identified in *Giardia* spp., *Plasmodium* spp., *Toxoplasma gondii,* or *Cryptosporidium parvum* (HMMer search using Pfam PF00335). Considering the role of TSPs in EV biogenesis in mammalian cells, it would be interesting to evaluate if the mechanism of EV formation differs among parasites that contain or lack TSPs within their genomes. For example, it has been demonstrated that TvTSP1 accumulates in Multivesicular Body (MVB)-like structures after *T. vaginalis* is exposed to vaginal epithelial cells (Twu et al. 2013). This accumulation of TvTSP1 in MVBs upon host cell exposure could suggest a role of the protein in cargo selection or biogenesis of exosomes. It is interesting to note that the expression of both TvTSP1 and TvTSP8 are up-regulated upon exposure of the parasites to the host cell (Coceres et al. 2015); indicating that they might be regulating the process of *T. vaginalis*: host interactions. On the other hand, TvTSP8 is highly expressed in adherent compared to less adherent *T. vaginalis* strains and overexpression of the surface-localized full-

length TvTSP8 promoted increased parasite aggregation (Coceres et al. 2015) (discussed in Sect. 4.1.1). This clumping phenotype is exhibited by some parasite strains, and correlates with their capacity to adhere to host cells (Lustig et al. 2013; Nievas et al. 2018a).

Besides tetraspanins, *T. vaginalis* EV proteomics analysis of EVs uncovered an enrichment for other proteins that have been implicated in host–parasite interactions and immunomodulation (Nievas et al. 2018a, 2020; Twu et al. 2013). Specifically, these include metalloproteases that are hypothesized to be involved in the infection process (Ma et al. 2011), BspA proteins that participate in host cell adherence (Handrich et al. 2019; Noel et al. 2010), the surface immunogen protein p270 (Alderete 1999), TvMIF that induces inflammatory responses (Twu et al. 2014), and proteins that are highly expressed in adherent strains (TVAG_020780 and TVAG_239650), albeit with unknown function (de Miguel et al. 2010; Gould et al. 2013). In order to fully understand the function of these proteins in EVs during infection, it would be interesting to analyze the protein content of the different vesicle populations in the presence and absence of host cells.

How host cells internalize EVs secreted by *T. vaginalis* is another important question. Recently, Rai and colleagues demonstrated that *T. vaginalis* exosomes fuse with and are internalized by host cells via caveolae and lipid raft-dependent endocytosis (Rai and Johnson 2019). Additionally exosomes were found to interact with glycosaminoglycans on the surface of host cells and to specifically bind heparan sulfate proteoglycans (HSPGs), indicating that HSPGs on host cells act as receptors that mediate the first step to internalize EVs (Rai and Johnson 2019). A ligand on the surface of EVs, named 4-α-glucanotransferase (Tv4AGT), was also identified to be critical for EV uptake (Rai and Johnson 2019). Interestingly, this protein is also present in the MV proteome (Nievas et al. 2018a), suggesting that mechanisms used to drive *T. vaginalis*: host interactions could be conserved among both types of vesicles. Future comparative analyses of the internalization pathways for different types of EVs will be necessary to fully understand the mechanisms involved in EV-mediated cell: cell communication.

4.3 *Host Immune Responses to* T. vaginalis *and Immune-Evasion by the Parasite*

Our understanding of how the host immune response to *T. vaginalis* proceeds, whether it effectively clears the parasite, and whether effective immunological memory forms against the parasite is certainly undercharacterized. However, researchers have gathered some information from in vitro studies and clinical correlations. While *T. vaginalis* is exclusively a human-infective pathogen, some preliminary murine models have also given insight into mammalian immune responses in vivo. Here we will discuss evidence of the innate and adaptive immune responeses to *T. vaginalis*, and findings that indicate immune-evasive behaviors of the parasite.

4.3.1 Cytokine Responses to *T. vaginalis*

Cytokines are important signaling proteins secreted from immune cells that are used to orchestrate immune responses. It has long been known that the pleitropic cytokine Interleukin-8 (IL-8) is released from human cells in response to *T. vaginalis* infection (Ryu et al. 2004; Shaio et al. 1994, 1995). Mice do not encode for the IL-8 gene, therefore it is not possible to test the effects of IL-8 in an experimental murine model. However, a cross-sectional study involving 65 women recently showed an increase in cervical IL-8 levels in *T. vaginalis*-exposed women compared to controls (Jarrett et al. 2015), giving in vivo validation to the in vitro observations of IL-8 secretion. Recent reports have also shown the cytokines IL-1β, CCL2, IL-17, IL-22, IFN-β, IL-6, RANTES, MIP-3α, TNF, and IL-23 to be associated with *T. vaginalis* infection either in vitro or in vivo (RN Fichorova et al. 2016; Fiori et al. 2013; Gu et al. 2016; Makinde et al. 2013; Mercer et al. 2016; Seo et al. 2014). Parasite-produced exosomes were also shown to induce IL-8 and IL-6 from host cells (Twu et al. 2013).

The cytokines released from cells that encounter *T. vaginalis* can give us clues about immune responses that form against the parasite. IL-1β, TNF, and IL-6 are all important cytokines that can get released systemically and act as fever-inducers (Garlanda et al. 2013). IL-1β, also indirectly promotes the recruitment of neutrophils, the first line of defense against most microbes, to the infection site (Slaats et al. 2016), and IL-8 does this directly. IL-1β, IL-6, and IL-23 are polarizing cytokines promoting the differentiation of T-helper 17 (Th17) cells (Z Chen and O'Shea 2008), pointing to a possible role of Th17 cells in *T. vaginalis* immunity, similar to *Giardia lamblia*, another mucosal-dwelling protozoan (Singer et al. 2019). RANTES, MIP-3α, and CCL2 are all involved in T-cell recruitment, and could thus be important for execution of T-cell responses against *T. vaginalis*. TNF and IL-1β are broadly inflammatory and consistent with the vaginitis and prostatitis associated with *T. vaginalis* infection.

As mentioned in Part 1, *T. vaginalis* is associated with increased spread of HIV. While the mechanism by which the parasite could promote enhanced HIV infection has not been well-characterized, recent data indicate that cytokines could play a role. *T. vaginalis* induces the inflammatory cytokines RANTES and MIP-3α (Fichorova et al. 2012), which are chemotactic ligands for the receptors CCR5 and CCR6 respectively. Therefore, *T. vaginalis* infection could promote the recruitment of CCR5+ and CCR6+ cells to the vaginal mucosa. As T-cells have high levels of expression of these two receptors, and CCR5 is a co-receptor for HIV entry, the parasite could increase the recruitment of HIV target cells to the vaginal mucosa, thus increasing susceptibility to HIV infection. Interestingly, RANTES and MIP-3α were found to be released only in response to *T. vaginalis* strains that harbor the symbiont *Trichomonasvirus* (discussed in Sect. 5.1) (Fichorova et al. 2012), indicating that *Trichomonasvirus* could faciliate increasing *T. vaginalis*-associated HIV contraction.

In fact, a majority of the cytokine induction by *T. vaginalis* appears not to be due to the parasite itself, but to *T. vaginalis* symbionts *Trichomonasvirus* or *Mycoplasma*

hominis (described in Sect. 5.1). When Fichorova and colleagues compared a panel of strains harboring *Trichomonasvirus* to strains without *Trichomonasvirus,* they observed that *Trichomonasvirus*+ strains were greatly enhanced in their ability to induce IFN-β, IL-8, IL-6, RANTES, MIP-3α and IL-1β in cervicovaginal epithelial cells (Fichorova et al. 2012), with strains lacking the virus showing almost no cytokine induction. Interestingly, this effect was enhanced in the presence of metronidazole (discussed in Sect. 7.1), a drug that kills *T. vaginalis*, presumably due to release of virions from dead parasites that could then more easily access host cell viral recognition receptors like TLR3. Similarly, Fiori and colleagues found that that *M. hominis* triggered the release of IL-8, IL-23, and TNF from a macrophage cell line, while *T. vaginalis* alone only stimulated small amounts of IL-1β. However, the *M. hominis*+ *T. vaginalis* consortium was able to induce greater amounts of cytokine than *M. hominis* or *T. vaginalis* alone, suggesting a synergism between the two microbes in inducing cytokines (Fiori et al. 2013). Other researchers also observed an *M. hominis*-dependent induction of IL-1β, IL-8, and IL-6 in primary human monocytes (Mercer et al. 2016). Each of these studies indicate that none, or very little cytokine is induced from the parasite alone. However, these studies may not be sensitive enough to detect cytokine induced from the parasite alone. Furthermore, during infection, the parasite likely elicits tissue damage, releasing dead-cell products that also likely stimulate cytokine induction. In addition, studies have not yet considered both symbionts at the same time. It would therfore be interesting to test a panel of strains containing both symbionts, or cleared of either, to better define the true contribution of each symbiont to cytokine stimulation. Data from Fiori and collagues, which used a *T. vaginalis* isolate naturally *Trichomonasvirus*- and *M. hominis*-free to stimulate a macrophage cell line, saw only weak activation of NF-κB and small amounts of IL-1β indicating weak immunostimulatory activity of the parasite in the absence of its symbionts. This might indicate that vaccines containing *T. vaginalis* immunogens need strong adjuvants since the parasite alone may not elicit strong responses.

4.3.2 *T. vaginalis* Recognition by the Innate Immune System

To determine how host immunity first perceives a *T. vaginalis* infection, an important question is whether the parasite stimulates Pattern-Recognition-Receptors (PRRs) on innate immune cells, and if so, which one(s). PRRs are essential for initial recognition of pathogens by the innate immune system and influence antimicrobial cellular responses and cytokine secretion by innate immune cells. The PRR Toll-Like Receptor −4 (TLR4) was posited to be triggered by *T. vaginalis*, because *T. vaginalis* was found to be less immunogenic in mutagenized mouse cells lacking TLR4 (Zariffard et al. 2004). However, it is not clear if these cells had other deficiencies besides lacking functional TLR4, or whether the parasites used in these experiments harbored *T. vaginalis* symbionts, which could confound the results. Furthermore, Fichorova and colleagues reported induction of cytokine secretion from purified *T. vaginalis* dominant surface lipoglycan (TvLG), in

cervicovaginal epithelial cells that do not express detectable levels of TLR4, and instead proposed a TLR4-independent mode of *T. vaginalis* recognition (RN Fichorova et al. 2006). The host lectin receptor, Galectin 3, has recently been shown to modulate IL-8 secretion through engagement of TvLG (Fichorova et al. 2016). Since host cell galectins are becoming appreciated as PRRs (Vasta 2012), the TvLG-Galectin interaction is an exciting area of future research into how *T. vaginalis* initially activates the innate immune system.

Furthermore, the observation that IL-1β is induced from host cells by *T. vaginalis* sparked interest in a potential role for the inflammasome in anti-*T. vaginalis* immune responses. IL-1β processing and secretion is largely driven by assembly of cytosolic PPRs called Nod-Like-Receptors (NLRs), that form multiprotein complexes called inflammasomes. Inflammasomes can activate caspase-1, which leads to cleavage of the pro-form of IL-1β to its bioactive form. IL-1β is then released from host cells as they die through pyroptosis (discussed in Sect. 4.1.3). Recently, researchers identified that *T. vaginalis* can activate the NLRP3 inflammasome, since knockdown or inhibition of caspsase-1 and NLRP3, decreased the amount of IL-1β observed in response to *T. vaginalis* (Gu et al. 2016; Riestra et al. 2019), and specifically the bioactive form (Riestra et al. 2019). Furthermore, Riestra and colleagues also detected the production of IL-1β in a murine model of *T. vaginalis* infection (Riestra et al. 2019), indicating that inflammasomes are activated by *T. vaginalis* in vivo. Additionally, a murine gut trichomonad, *Tritrichomonas muris* was observed to induce an inflammasome- dependent program of immunity in vivo (Chudnovskiy et al. 2016). However, it remains to be investigated, whether inflammasome activation contributes to control of *T. vaginalis* infection or whether it leads to chronic inflammation. It will also be interesting to determine whether *T. vaginalis* strains vary in their potency to activate inflammasomes.

4.3.3 Innate Immune Responses to *T. vaginalis*

While the innate immune response to *T. vaginalis* is not fully characterized, in vitro work has shown that phagocytes (mainly neutrophils) and the complement system are likely to be important for immune clearance of the parasite. As mentioned above, IL-8, which has functions in neutrophil recruitment, is the most abundant cytokine induced by *T. vaginalis* in in vitro assays, and has been found at the site of infection (Cauci and Culhane 2007; Lazenby et al. 2013; Mercer et al. 2016; Shaio et al. 1994, 1995). In 1980, researchers observed that *T. vaginalis* is killed by neutrophils in vitro after they swarm the parasite in the presence of serum, indicating that neutrophils work in aggregate, cooperating to kill *T. vaginalis* (Rein et al. 1980). Researchers also found that supernatants from *T. vaginalis* cultured with epithelial cells induce neutrophil migration over a filter (Seo et al. 2014), a proof-of-principle experiment that neutrophils chemotax towards *T. vaginalis* infection products. Collectively, these results support a crucial role for neutrophils in elimination of *T. vaginalis*.

More recently, researchers have sought to determine the specific cellular mechanism(s) that neutrophils use to kill *T. vaginalis*. Neutrophils are known to have

3 classical modes of killing pathogens, all of which involve the neutrophil granules, which are vesicles present in neutrophils that contain toxic substances like pore forming toxins, reactive oxygen species, and enzymes (Kolaczkowska and Kubes 2013). The first mode is phagocytosis, in which neutrophils engulf pathogens whole. The vacuole containing the pathogen then fuses with lysosomes or neutrophil toxic granules to kill the pathogen. The second mechanism is extracellular degranulation, in which the toxic granules are exocytosed to intoxicate pathogens extracellularly. The third mechanism is by using Neutrophil Extracellular Traps (NETs), a process termed NETosis, in which the neutrophil ejects its DNA, producing an unraveled NET studded with the contents of toxic granules so that ensnared pathogens are intoxicated by granule contents. Generally, phagocytosis and extracellular degranulation are very rapidly employed (within minutes), while NETosis is a late-stage killing mode, possibly employed by neutrophils as a last-ditch effort if the other two mechanisms have failed.

When researchers tested which mode of killing neutrophils employ against *T. vaginalis*, they found that the parasite was killed very rapidly and in a contact and engulfment-dependent process, but that as opposed to phagocytosis, neutrophils mainly kill *T. vaginalis* by taking piece-meal bites of the parasite surface until it dies (Mercer et al. 2018). Such cellular "nibbling" is termed trogocytosis (trogo = to nibble), contrasted with traditional phagocytosis (phago = to eat), in that the target does not get engulfed whole (Ralston 2015a). It is not yet known whether the neutrophil toxic granules are involved in generating the nibbles upon cell-cell contact, or whether the neutrophil has other mechanisms to degrade the parasite surface. However, since neutrophil trogocytic killing of *T. vaginalis* was almost completely inhibited when neutrophils were pre-incubated with a serine protease inhibitor, it is hypothesized that serine proteases present in neutrophils may contribute to trogocytic killing (Mercer et al. 2018). In addition, since human serum is required for neutrophil trogocytic killing of *T. vaginalis* (Mercer et al. 2018), it is likely that complement proteins, reactive proteins found in serum that can mediate pathogen killing, mediate neutrophil binding to *T. vaginalis* to initiate trogocytosis. Complement proteins have also been shown to directly kill some strains of the parasite (Gillin and Sher 1981). While complement proteins and trogocytosis by neutrophils are likely to be rapid killing mechanisms used against *T. vaginalis*, it is not yet known whether neutrophils can also kill the parasite using NETosis, at later time points.

4.3.4 Adaptive Immune Responses to *T. vaginalis*

It is not known if, or how often, effective immunological memory to *T. vaginalis* develops in hosts. Antibodies that recognize the parasite or its components, have been detected in patients, indicating that an adaptive immune response is formed. For example antibodies reactive against TvMIF have been detected in patients (Kaur et al. 2008; Paintlia et al. 2002; Twu et al. 2014) and antibodies against the cysteine proteases TvCP2, TvCP4, TvCPT, and TvLEGU-1 have also been identified in

vaginal lavages and in serum collected from *T. vaginalis*-infected women (Alvarez-Sanchez et al. 2000; Hernandez-Gutierrez et al. 2004; Mendoza-Lopez et al. 2000; Ramon-Luing et al. 2010). Additionally, both IgG and IgA antibodies specific to the dominant surface lipoglycan TvLG (Bastida-Corcuera et al. 2013), have been found in both the blood and vaginal secretions of infected patients. Furthermore, a serological study conducted on a cohort of patients in Vietnam described the kinetics of circulating antibodies in response to *T. vaginalis*. Successful treatment of the infection resulted in a relatively fast decline of ciruculating antibody titers, while antibody persistence was related to possible drug resistance, or alternatively to "ping-pong" reinfection by untreated sexual partners, thus suggesting that the humoral response to *T. vaginalis* might be transient and not entirely protective (Ton Nu et al. 2015). In support of this, several studies have noted higher rates of rebound trichomoniasis when partners were not treated, further indicating that immunological memory may not effectively form against *T. vaginalis* (Forna and Gulmezoglu 2003). Interestingly, adverse pregnancy outcomes are associated with lower vaginal IgG, indicating that IgG could be protective, without inciting immunopathology (Bastida-Corcuera et al. 2013). Therefore, future prophylactic vaccines may require boosters or strong adjuvants to achieve protection.

Furthermore, neutrophil trogocytic killing of *T. vaginalis* is dependent on human serum, and roughly 50% of neutrophil trogocytic activity was inhibited in the presence of Fc proteins (Mercer et al. 2018), which compete for antibody receptors. It is therefore likely that antibodies play a role in neutrophil killing of *T. vaginalis*, highlighting that neutrophil trogocytosis is part of the adaptive immune process against *T. vaginalis* as well.

CD4+ T-cells are also thought to be recruited to the site of infection, as T-cell recruitment cytokines are induced from other cells by the parasite (Fichorova et al. 2012) and CD4+ T-cell levels increase in the vaginal mucosa after parasite inoculation in a mouse model (Smith and Garber 2015). How T-cells respond to *T. vaginalis* is not known, and which T-helper cell subsets differentiate and help shape immune responses to *T. vaginalis* has not been formally tested, however IL-17 and IL-22 responses have been reported as elevated in *T. vaginalis* + women compared to non-infected controls (Makinde et al. 2013), indicating that Th17 responses could play a role. Since major functions of a Th17 adaptive response is promotion of neutrophil recruitment and combating extracellular pathogens, this is a logical hypothesis. Of note, the presence of *T. vaginalis'* symbionts (discussed in Sect. 5.1) will likely also influence T-cell polarization.

4.3.5 *T. vaginalis* Subversion of Host Immune Responses

The relatively high percentage of partner re-infection observed in some studies (Forna and Gulmezoglu 2000), and the long-term persistence of some infections, indicates that many strains of *T. vaginalis* are able to evade the immune response. Studies of neutrophil trogocytic killing show that on average, five neutrophils were found to surround one trichomonad during killing in vitro (Mercer et al. 2018).

Therefore, it is highly likely that the routinely observed behavior of *T. vaginalis* forming microcolonies (i.e. clumping) (Coceres et al. 2015; Nievas et al. 2018b) discussed in Sect. 4.1, could serve as a neutrophil-evasive behavior, making it challanging for neutrophils to surround individual trichomonads. Additionally, *T. vaginalis* has been reported to induce apoptosis of neutrophils in vitro, indicating that killing of neutrophils could be a strategy that some strains use in some circumstances to evade killing (Song et al. 2008). Furthermore, *T. vaginalis* produces cysteine proteases that show the ability to degrade antibody proteins in vitro, suggesting that the parasite could evade trogocytosis and other modes of antibody-dependent killing (Hernandez et al. 2014; Provenzano and Alderete 1995).

As mentioned above, while neutrophils employ trogocytosis as an early-stage killing mechanism against *T. vaginalis*, it is not yet known whether neutrophils can also kill *T. vaginalis* using NETosis, at later time points. However it is interesting that a DNAse was recently discovered to be part of the *T. vaginalis* constitutive secretome (Stafkova et al. 2018), indicating that the parasite could harbor a strategy to evade killing by NETosis. Furthermore, the *T. vaginalis* symbiont *M. hominis* (discussed in Sect. 5.1) was recently discovered to express a surface nuclease, MH_0730, which was also found to be capable of degrading DNA (Cacciotto et al. 2019). In the same paper, researchers showed that *M. hominis* could also degrade NETs in vitro (Cacciotto et al. 2019). Therefore, one interesting possibility is that while *M. hominis* may make *T. vaginalis* more immunogenic because it induces more cytokine secretion, it may also help the parasite evade killing by neutrophil NETosis.

Another potential mechanism of immune-evasion could be the cytolytic properties of the parasite. *T. vaginalis* was shown to be able to kill lymphocytes in vitro (Mercer et al. 2016). Interestingly, strains that were more lytic to epithelial cells (Lustig et al. 2013), were also found to be lytic to lymphocytes, indicating that these strains thought to be more pathogenic could also evade adaptive immunity by killing its cellular effectors. Furthermore, this lytic activity preferentially targeted B-cells, the producers of antibody, further supporting the hypothesis that *T. vaginalis* employs evasive behaviors against antibodies.

Interestingly, *T. vaginalis* has also been found to make a cytokine mimic, and a mimic of leukotriene, a chemical mediator of inflammation. TvMIF, a homolog of human MIF (Macrophage Migration Inhibitory Factor), is secreted from the parasite, and can bind the human MIF receptor, increasing host inflammatory cytokine production (Twu et al. 2014). It is not known whether TvMIF-induced inflammation promotes *T. vaginalis* clearance, or contributes to immune subversion through distraction, as previously observed for a MIF ortholog encoded by the unicellular parasite *Plasmodium berghei* (Sun et al. 2012). Better understanding of effective immune responses against the parasite will help to elucidate this. However, TvMIF was found to increase *T. vaginalis* survival by inhibiting apoptosis in parasites under nutrient stress (Chen et al. 2018), indicating that TvMIF could play an indispensable role in *T. vaginalis* survival during infection, potentially overshadowing any negative role associated with enhanced immune-recognition. *T. vaginalis* is also thought to secrete a homolog of Leukotriene B4 (Nam et al. 2012), which has activity in

neutrophil recruitment. It is not yet clear what function *T. vaginalis*-derived LTB4 has in infection.

EVs secreted by *T. vaginalis* (discussed in Sect. 4.2) likely also contribute to immune subversion. While exosomes secreted by *T. vaginalis* were shown to induce inflammatory cytokine release from human cells (Twu et al. 2013) indicating that they can mobilize immunity against *T. vaginalis*, several reports have also shown evidence that *T. vaginalis* exosomes could be immuno-suppressive. Twu and colleagues demonstrated that pre-treatment with *T. vaginalis* exosomes reduced subsequent IL-8 responses to the parasite itself in vitro (Twu et al. 2013), indicating that exosomes could "prime" more distal parts of the vaginal mucosa to be tolerant toward the parasite in vivo. Moreover, another group found that pre-treatment of mice with *T. vaginalis* exosomes reduced IL-17 secretion and gross inflammation scores during infection, indicating that exosomes could promote reduced inflammation towards the parasite (Olmos-Ortiz et al. 2017). Moreover, two reports found that exosomes or secretory products from *T. vaginalis* increased IL-10 production (Olmos-Ortiz et al. 2017; Song et al. 2015), and another report found IL-10 induction from bovine cells with the bovine vaginal trichomonad *Tritrichomonas foetus* (Vilela and Benchimol 2013). Since IL-10 is an anti-inflammatory cytokine promoting tolerance to microbes, *T. vaginalis* exosome induction of IL-10 production could be another mechanism by which the parasite evades immune clearance. *T. vaginalis* exosome generation could therefore be a mechanism that the parasite uses to dampen host immunity, allowing it to establish infection similar to the secretion of virulence factors and vesicles by bacteria (Kuehn and Kesty 2005; Kulp and Kuehn 2010). It is not clear whether anti-inflammatory properties of exosomes could also have beneficial anti-inflammatory roles for the host, or whether they merely thwart effective clearance of the parasite.

T. vaginalis can modify the local environment aiding its ability to attach to and lyse cells. In parallel, the immune response is also mobilized to control infection. The spatial and temporal nature of these processes and how they influence one another now remains to be further explored in vivo. Furthermore, during host colonization *T. vaginalis* also has important interactions with the microbes residing in the urogenital tract, as described next.

5 Part 5: *T. vaginalis* as a Member of a Rich Microbial Ecosystem

A real view of host-parasite interactions, particularly for extracellular pathogens, would be incomplete without including the other microbes indigenous to the site of infection and colonization. Interestingly, in addition to members of the urogenital microbiome, *T. vaginalis* also carries its own microbial symbionts (bacteria and viruses). We will first discuss the bacterial and viral partners of *T. vaginalis*, that often hitch-a-ride during infection and are undoubtedly important contributors to

infection biology. We then look further outward, considering *T. vaginalis'* interaction with members of the vaginal microbiome and how the parasite may participate in driving dysbiosis and/or exploit this microbial shift for its own benefit.

5.1 Symbionts of T. vaginalis

A distinctive and peculiar feature of *T. vaginalis* is its unique ability, among human pathogenic protists, to establish symbiotic relationships with eubacteria belonging to the *Mycoplasma* genus (Dessi et al. 2019), namely *Mycoplasma hominis* and *Candidatus Mycoplasma girerdii.*

In the complex field of interspecies relationships found in the microbial world, two paradigmic protists for symbiotic relationships with bacteria and viruses have emerged: the obligate human parasite *T. vaginalis* and the free-living amoeba (FLA) *Acanthamoeba spp*. While the potential role of *Acanthamoeba* as an environmental reservoir and a "biological gym" for intracellular bacterial human pathogens has been the subject of intense investigations (Balczun and Scheid 2017), research on the impact of *T. vaginalis'* symbionts on its biology is still unexplored in many aspects, despite the first description of the biological association between *T. vaginalis* and *Mycoplasma hominis* over 20 years ago (Rappelli et al. 1998). This is the first reported case of a biological association between two human microbial pathogens with an obligate parasitic lifestyle, which are able to cause independent diseases in the same anatomical region. A considerable number of studies ever since, have investigated the *T. vaginalis* and *M. hominis* association rates in clinical isolates from different geographical locations, highlighting a remarkable variability (5% to 89%) (R Fichorova et al. 2017).

5.1.1 *Mycoplasma hominis*

M. hominis is a human sexually transmitted bacterium belonging to the group of *Mollicutes*, a Class of atypical bacteria with a small genome size, lacking a cell wall and several metabolic pathways. These unique characteristics are reflected by the strong dependency of mycoplasmas on host cell environment: indeed, these microorganisms depend metabolically on a host cell to survive and reproduce, thus they have evolved an obligate parasitic lifestyle. The complete genome sequences of several *Mollicutes* revealed the genetic basis of such dependence. Mycoplasmas lack genes necessary for the biosynthesis of amino acids and nucleic acid precursors, and are thus unable to synthesize essential macromolecules. *M. hominis* utilizes arginine as an energy source through the Arginine dihydrolase (ADH) pathway, which leads to ATP production via substrate-level phosphorylation (Razin et al. 1998).

Genital infections by *M. hominis* are associated with bacterial vaginosis and with a number of symptoms, but the pathogenicity mechanisms of this bacterium are still unclear. *M. hominis* can be isolated from the genital tract of both symptomatic and

asymptomatic individuals and it is considered a commensal microorganism of the genital tract that can act as a pathogen in certain circumstances. Nevertheless, there is evidence that *M. hominis* may play an important etiologic role not only in genital tract diseases of both men and women, but also in extragenital infections. The mechanisms by which *M. hominis* exerts its pathogenic effects still need to be elucidated in detail: only a limited number of proteins have been associated with virulence. P80, P50/vaa and OppA were shown to play a role in adhesion to host cells (Henrich et al. 1996; Hopfe et al. 2004; Kitzerow et al. 1999). Interactions of *M. hominis* with host immunity has also received attention: mycoplasmal lipoproteins are able to induce IL-23 production by dendritic cells (Goret et al. 2016), and a specific predicted surface lipoprotein (MHO_0730) was suggested to have a role in evasion from innate immune response, through the disruption of Neutrophil Extracellular Traps (NETs), as discussed in Sect. 4.3.3 (Cacciotto et al. 2019).

A first characterization of *T. vaginalis: M. hominis* symbiotic association showed how the bacterium is able to reside and multiply within trichomonad cells (Fig. 5a) (Dessi et al. 2005), thus gaining a niche which may allow protection from immune responses and antibiotic treatments. *T. vaginalis* could also transmit the mycoplasmal infection to a human cell line, and to naturally *M. hominis*-free trichomonad isolates (Rappelli et al. 2001). Additionally, Thu and colleagues investigated a potential mechanism by which *T. vaginalis* may function as a protective niche to transport virulent *M. hominis* to human cell cultures (Thi Trung Thu et al. 2018). They treated *Mycoplasma*-infected trichomonad cells with metronidazole, which led to *T. vaginalis* death and subsequent release of viable bacteria from *T. vaginalis,* which in-turn infected cultured human cells. A similar process might also occur in vivo. Together, these findings suggest a role for *T. vaginalis* to serve as a "Trojan horse" for *M. hominis*.

As described previously, the current model of trichomoniasis pathogenesis is driven both by the direct cytopathic effect exerted by *T. vaginalis* through cytoadhesion and expression of cytotoxic proteins, and by the host inflammatory response which can contribute to mucosal tissue damage (Mercer and Johnson 2018). The association of *T. vaginalis* with *M. hominis* affects both aspects of *T. vaginalis* immunopathogenesis. Vancini and collaborators showed that *M. hominis*-infected *T. vaginalis* displayed an increased ability to induce epithelial cellular damage in vitro, compared to uninfected trichomonads (Vancini et al. 2008). Similarly, researchers found that *M. hominis* upregulates *T. vaginalis* haemolytic activity in vitro (Margarita et al. 2016). Lysis of erythrocytes by *T. vaginalis* occurs in vivo, and the heamolytic activity and the consequent acquisition of nutrients, especially iron, have been suggested to be responsible for the exacerbation of symptoms observed during and following menstruation (Lehker et al. 1990). However, no increased lysis of leukocytes was observed when *T. vaginalis* harbored *M. hominis* (Mercer et al. 2016), indicating that the ability of *M. hominis* to influence *T. vaginalis'* host-cell killing behavior may vary based on the host cell type or cell death pathway triggered (discussed in Sect. 4.1.3). Regarding the inflammatory potential of *M. hominis* however, the microbial symbiosis induced an increase in inflammatory cytokines produced by host immune cells, as discussed in Sect. 4.3.1

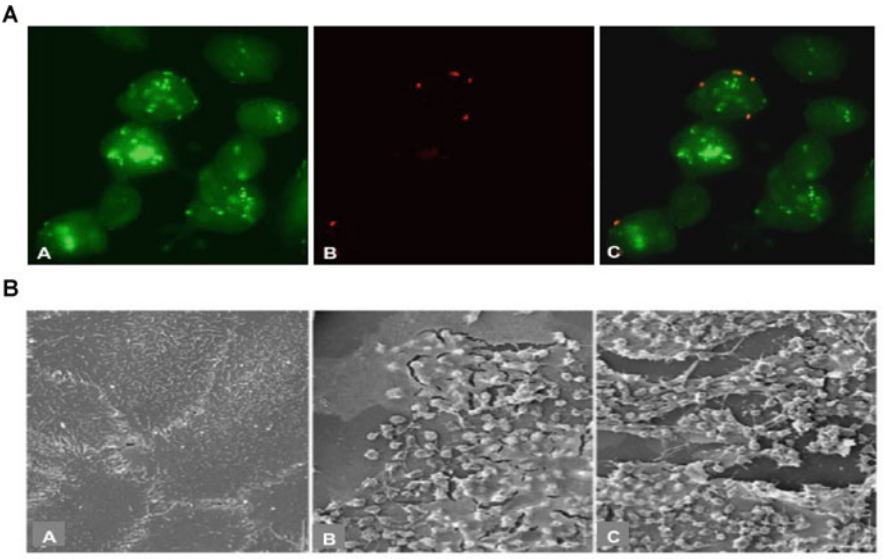

Fig. 5 *T. vaginalis* association with *Mycoplasma hominis.* (**a**) Double-immunofluorescence micrographs depicting the cellular localization of *M. hominis* infecting *T. vaginalis.* Panels A to C represent the same area of a protozoan monolayer. (A) FITC fluorescence showing extracellular and intracellular mycoplasmas.(B) Rhodamine fluorescence showing mycoplasmas that are extracellularly located. (C) Superimposed images of panels A and B indicating the localization of extracellular (red) and intracellular (green) mycoplasmas. (**b**) SEM image of *T. vaginalis* harboring *M. hominis* in contact with human vaginal epithelial cells (hVECs). Notice the damage to the vaginal epithelial cells comparing uninfected hVECs control (A) and after 1 hr of interaction with *T. vaginalis* and its associated endosymbiont *M. hominis* (B, C). Scale bar=20μm. Figure (**a**) was adapted from Copyright © American Society for Microbiology, [Dessi et al. Infect Immun, 73(2), 2005, 1180-6, DOI 10.1128/IAI.73.2.1180-1186.2005]. Figure (**b**) was adapted by permission from the publisher: Springer Nature, Eur J Clin Microbiol Infect Dis, https://link.springer.com/article/10.1007%2Fs10096-007-0422-1, *Trichomonas vaginalis* harboring *Mycoplasma hominis* increases cytopathogenicity in vitro, Dessi et al. Copyright 2008

(Fiori et al. 2013; Mercer et al. 2016). Thus *M. hominis* could play a role in the increased risk of HIV acquisition and cervical cancer progression associated with trichomoniasis, since both are likely linked to inflammation (McClelland et al. 2007; Sutcliffe et al. 2012; Yap et al. 1995). Furthermore, *T. vaginalis* and *M. hominis* are both associated with an increased risk of adverse pregnancy outcomes, which are also associated with increased inflammation (Gomez-Lopez et al. 2010; Pararas et al. 2006; Petrin et al. 1998). Therefore, we hypothesize that the upregulation of the host inflammatory response resulting from protist-mycoplasma symbiosis might have an impact on clinically important morbidity associated with trichomoniasis.

Besides the contribution of the *T. vaginalis-M. hominis* symbiotic relationship to the infection biology of both microorganisms, the possible effect of *M. hominis* on the metabolism and physiology of the protozoan has also been investigated. *T. vaginalis* and *M. hominis* are both equipped with the ADH metabolic pathway,

which uses arginine as an energy source for ATP production. While this pathway contributes up to 10% of the ATP in *T. vaginalis* (Yarlett et al. 1996), the energy metabolism of *M. hominis* entirely relies on ADH (Pereyre et al. 2009). Morada and colleagues investigated the arginine metabolism in *M. hominis*-infected *T. vaginalis,* and described how the production of ornithine and putrescine, two key intermediates in this biochemical pathway, are increased 16 and 3 fold respectively, compared to *Mycoplasma*-free trichomonad cells (Morada et al. 2010). This phenomenon is accompanied by an increase in arginine consumption and has two important consequences. Firstly, *M. hominis* increases growth rates of *T. vaginalis*, in an arginine-metabolism dependant manner (Margarita et al. 2016). Secondly, the upregulation of arginine scavenging may contribute to the survival strategies of these pathogens in the host. Arginine is the substrate for the production of nitric oxide, a known compound with microbicidal activity produced by host macrophages (Dillon et al. 2002). Thus reduced nitric oxide levels may negatively affect host defense, and the metabolic synergy between *T. vaginalis* and *M. hominis* may therefore confer a fitness advantge during infection, to both microbes.

5.1.2 *Candidatus Mycoplasma girerdii*

A 16S rRNA-based metagenomic analysis of the vaginal microbiota in *T. vaginalis*-infected and uninfected patients (Martin et al. 2013), led the authors to serendipitously identify a potential new symbiont: a previously unknown *Mycoplasma* species strictly associated with *T. vaginalis* infection. Initially dubbed *Mnola*, this unknown *Mycoplasma* was further characterized through the reconstruction of its genome (Fettweis et al. 2014). The authors proposed the name "*Candidatus Mycoplasma girerdii"* for this microorganism, still to be officially recognized as a new species. *Ca. M. girerdii* is still a non-culturable bacterium, and only a limited number of studies have taken into account its presence in patients (Costello et al. 2017; Ioannidis et al. 2017; Masha et al. 2018). Despite the detection of putative virulence genes in the genome sequence (Fettweis et al. 2014), the hypothetical influence of *Ca. M. girerdii* on *T. vaginalis* pathogenesis is still unknown.

5.1.3 *Trichomonasvirus*

In addition to the symbiotic relationships with *M. hominis* and *Ca. M. girerdii*, *T. vaginalis* can be infected by a dsRNA virus, *Trichomonasvirus*. *T. vaginalis* is not unique, among parasitic protozoa, in harboring viruses (Wang and Wang 1991). The presence of non-fragmented dsRNA of viral origin in *T. vaginalis* isolates was first established by Alice and C.C. Wang back in 1986 (Wang and Wang 1986). Since then, many studies focused on the extent of this viral infection in *T. vaginalis* clinical isolates, and on the molecular characterization of *Trichomonasvirus* (Goodman et al. 2011). *T. vaginalis* isolates are commonly infected by four different viral species (*Trichomonasvirus* I–IV), which can concurrently be found in the same *T. vaginalis*

isolate. The rates of detection of *Trichomonasvirus* show a high degree of variability globally, ranging from 17.4% in a study conducted in Iran (Heidary et al. 2013) to 81.9% detected in South Africa (Weber et al. 2003). The reasons for the extent of this variability could be due to the different geographical settings, to differences in the detection techniques used, or the small population sizes subjected to analysis. We predict that until now, the potential impact of the presence of *Trichomonasvirus* in *T. vaginalis* isolates on human pathology has been underestimated. Indeed, work in this specific field is sparse and open to interpretation. As described in depth previously in this chapter, adhesion to the mucosal surface is an initial critical step in *T. vaginalis* virulence. Fraga and colleagues, observed that *Trichomonasvirus*-harboring *T. vaginalis* strains isolated in Cuba showed increased adherence levels in vitro and pathogenicity in patients, evaluated as severity of symptoms (Fraga et al. 2012). This observation could be explained by the presence of the virus itself or, alternatively, by a predisposition to viral infection by more inherently pathogenic strains. The most significant impact of *Trichomonasvirus* on *T. vaginalis* pathogenic processes is likely on the host immune response, as discussed in Sect. 4.3.1.

The discovery of microbial symbionts co-existing with *T. vaginalis* opens many exciting questions focused on a more nuanced understanding of infection biology in the context of the metabolic and immunological contributions that the symbionts make to the parasitic unit. In many ways, infection with strains harbouring any or all of these symbionts, could be considered a polymicrobial infection, or a potential evolutionary unit—"holobiont" (O'Malley 2017). However, many questions that these complexities raise remain to be answered. These include: (1) What are the specific mechanisms by which *M. hominis/Ca. M. girerdii/Trichomonasvirus* influence immunopathogenesis? (2) Do the symbionts have an impact on clinical therapy for trichomoniasis? (3) What are the evolutionary advantages and implications for all the actors involved in this polymicrobial consortium which could at least partially explain the strict relationships observed? (4) Are these symbiotic relationships still in infancy, from an evolutionary point of view?

5.2 T. vaginalis *and the Host Microbiome*

In addition to carrying its own symbionts, *T. vaginalis* colonizes two distictive sites of the human body that are naturally populated by commensal microorganisms (i.e. the host microbiome). Therefore, it is highly expected that the interaction of *T. vaginalis* with these indigenous microorganisms might be influential to the outcomes of infection and disease. The urinary and reproductive tracts, sites of *T. vaginalis* infection, are very distinctive anatomical and physiological environments. Their mucosal surfaces are colonized by niche-specialized communities of microorganisms that display specificity to a location within these tracts and to the sex. Even deep organs of the urogenital tract in men and women, once thought to be sterile, have now been found to harbor a specific microbiota (Bao et al. 2017; Javurek et al. 2016; Moreno and Simon 2019). The microbial density in both urinary

and reproductive tracts tends to decrease when ascending from the lower to the higher region of the tracts; possibly helping preserve essential functions like reproduction (Moreno and Simon 2019). The lower urogenital tract (i.e. habitat of *T. vaginalis*) in particular, is densely colonized by commensal microorganisms.

No other site of the urogenital tract is as densely colonized by microorganisms as the ectocervix and vagina, with up to 10^7–10^8 bacteria per ml recovered from cervicovaginal secretions of women at reproductive age (Redondo-Lopez et al. 1990). Like no other female mammal, humans have developed a complex relationship with their cervicovaginal microbiome (CVM) that has a direct impact on their reproductive health (Anahtar et al. 2018; Younes et al. 2018). The higher incidence of trichomoniasis in women than men can be attributed to a combination of factors in men, including insensitive diagnosis and presumptive treatment, milder symptoms, and shorter duration of infection (Kissinger 2015). For these reasons, research on the interaction of *T. vaginalis* with the microbiota has been particularly focused on the CVM.

5.2.1 Mutualism Between the Human Cervicovaginal Microbiome and Host

The histophysiology of the cervicovaginal mucosa and the CVM change with age (Younes et al. 2018). The mucosal lining, consisting of a stratified squamous non-keratinized epithelium, reaches its maximum thickness at reproductive age. While the microbial community of the pre-menarchal (before first menstruation) and menopausal vagina may resemble the one of the skin or gut, the composition and dynamics of the CVM at reproductive age is unique. Specifically, reproductive-age CVM is rich in lactobacilli in most women, with almost exclusive predominance of a single vaginal species of *Lactobacillus*. In an ethnically-restricted cohort of asymptomatic women of reproductive age, ~75% were found to harbour a lactobacilli-rich CVM, which was classified into community state types (CSTs) depending on the predominant species of *Lactobacillus*: CST-I for *L. crispatus*, CST-II for *L. gasseri*, CST-III for *L. iners* and CST-V for *L. jensenii,* while CST-IV which was characterized by low abundance or absence of lactobacilli but diversified bacterial species (Ravel et al. 2011). However, it is important to note that on average, the microbiome literature presents a biased representation of ethnic and racial groups with emphasis on Caucasians, African-Americans and Hispanics.

A compelling mutualistic relationship between the human host and vaginal lactobacilli develops during reproductive age. Estrogen induces proliferation of the cervicovaginal cells and glycogen production, while progesterone promotes cell lysis and glycogen release. The consumption of glycogen by lactobacilli leads to the natural acidification of the vagina, due to release of high amounts of lactic acid. In addition to acidification and secretion of other anti-microbial substances, lactobacilli support the host by enhancing epithelial barrier function and stimulating innate immune responses (Anahtar et al. 2018; Pekmezovic et al. 2019; Younes et al. 2018). Strikingly, this is a human-specific phenomenon, not seen even among our

closest primate relatives, and is driven by the natural fluctuation of hormones across the menstrual cycle (Younes et al. 2018). The vaginal microbiomes of nonhuman primates display species specificity following phylogeny; in spite of this, not even our closest primate relatives exhibit a vaginal microbiome with *Lactobacillus* dominance (Yildirim et al. 2014a). Longer gestation periods and a smaller pelvic oulet in humans than other primates may have acted as evolutionary drivers of this recent friendship between humans and their *Lactobacillus*-dominant vaginal microbiota (Yildirim et al. 2014a). A microbially acidified vagina provides better pregnancy outcomes, in addition to protecting both mother and fetus against infections.

5.2.2 Correlates of Typical Cervicovaginal Microbiome with Eubiosis and Dysbiosis

Despite the dominance of lactobacilli, a considerable fraction of asymptomatic, childbearing age women (~25%) harbour a species-diversified CVM (CST-IV); which virtually excludes lactobacilli and instead is composed of mostly anaerobic bacteria with the predominance of species commonly found in bacterial vaginosis (BV). These bacterial species include *Gardnerella vaginalis*, *Prevotella*, *Megasphera*, *Snethia* and *Atopobium vaginae* (Ravel et al. 2011). Lactobacilli are keystone commensals contributing to eubiosis of the vagina (i.e. the homeostasis of the vaginal biome). Lactobacilli are certainly known to confer protection and optimal immune-physiological function of the vagina; however they do not always necessarily define vaginal health (Amabebe and Anumba 2018), due to several considerations. Firstly, the lactobacilli-low CST-IV and BV are more common among Black and Hispanic women than Caucasians for example. Secondly, the CVM of an individual is dynamic; its composition varies temporarily with the menstrual cycle and sexual activity, among many other factors. Finally, not all lactobacilli provide host protection to the same level. The protective role of *Lactobacillus iners*, for instance, is actually questionable. A *L. iners*-dominated vagina (CST-III) is less stable, more prone to transition and more associated with dysbiosis than CST-I and CST–V (*L. crispatus* and *L. jensenii*, respectively) (Amabebe and Anumba 2018). A *L. gasseri*-dominated vagina (CST-II) is the most stable of all, rarely transitioning to dysbiosis (Boris and Barbes 2000; Gajer et al. 2012). While most vaginal lactobacilli absolutely contribute to eubiosis of the vagina, the hallmarks of a 'healthy vagina' cannot be established without a real understanding of gene-environment interactions (Amabebe and Anumba 2018).

5.2.3 Correlates of Cervicovaginal Microbiome with Trichomoniasis

Clinically, trichomoniasis is often associated with vaginal dysbiosis, characterized by a reduction of vaginal lactobacilli, which is thought to contribute to some of the symptoms of the infection. Additionally, patients frequently display an intermediate

Nugent score, indicating diversified bacterial morphotypes (Petrin et al. 1998). However, only recently did cross-sectional surveys using metagenomics show a clear association of trichomoniasis with CST-IV. One study has identified that 8 out of 11 cases of asymptomatic *T. vaginalis* infections, among 396 women of child-bearing age, were accompanied by the CST-IV microbiome (Brotman et al. 2012). In a separate study, samples stratified by Nugent score were examined (Martin et al. 2013). As expected, women with intermediate Nugent score were more likely to have *T. vaginalis* infection. Importantly, *T. vaginalis* infected women with an intermediate Nugent score carried a CVM that closely mirrors that of BV (Martin et al. 2013). This finding reinforces the early epidemiological finding that women with BV have higher chances to acquire trichomoniasis (Rathod et al. 2011). Together, these two studies (Brotman et al. 2012; Martin et al. 2013) have brought out compelling evidence that either a specific CVM facilitates *T. vaginalis* infection or that the parasite itself could possibly shape the microbiota towards CST-IV, perhaps favoring its survival or transmissibility. Although these studies agree upon a strong association between trichomoniasis and a species-diversified CVM, they should be interpreted with a note of caution. For example, women of Black and Hispanic ethnicities were largely represented in both studies. In addition to limited sample sizes, neither study took into consideration the temporal stability of the *T. vaginalis*-association with the microbiome. Ideally, longitudinal studies from variable ethnicities will be necessary to confirm the association of *T. vaginalis* with the CST-IV type of CMV, and to determine the drivers of cause-and-effect (i.e. whether *T. vaginalis* drives CST-IV, or CST-IV predisposes to *T. vaginalis* infection) (Fig. 6a).

Nonetheless, the correlation of trichomoniasis with a specific type of microbiota, which excludes lactobacilli and resembles BV, is at least intriguing. This possible unique inter-domain relationship (protozoa and bacteria) in the vagina evokes basic principles of microbial ecology (Coyte et al. 2015). It suggests that *T. vaginalis* and indigenous vaginal bacteria are competitors in this specific ecological niche. If a cause-and-effect can be established between microbiota and *T. vaginalis*, then niche competition or tradeoffs (Bauer et al. 2018; Limberger and Wickham 2011; Smith et al. 2018; Yawata et al. 2014) between *T. vaginalis* and vaginal commensals might be critical for infection and potentially decisive to its outcomes. Lactobacilli-mediated host defense may impact the evolution of *T. vaginalis* virulence (Ford et al. 2016). Competitive exclusion, where protozoa wins over lactobacilli, could potentially drive vaginal dysbiosis. Meanwhile, competition-colonization tradeoffs could explain a preferential coexistence of *T. vaginalis* with the species-diversified CST-IV. Applying this knowledge to the development of novel treatments will be possible, if we understand the underlying principles and mechanisms that support these microbial interactions in the vagina. Just recently, vaginal microbiome transplantation was shown to be relatively successful for women suffering from intractable and recurrent BV (Lev-Sagie et al. 2019).

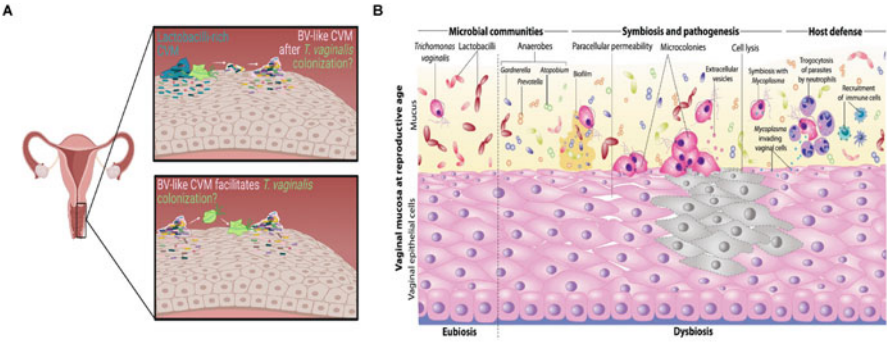

Fig. 6 *T. vaginalis* interaction with the cervicovaginal microbiome (CVM). (**a**) Interplay of *T. vaginalis* and the CVM. (Top panel) *T. vaginalis* infection may lead to the loss of predominant *Lactobacillus* species (turquoise bacteria) in the cervicovaginal microbiome (CVM) and promote increased vaginal colonization by a mixed and largely anaerobic bacterial population, similar to that associated with bacterial vaginosis (BV-CVM, mixed color bacteria). (Bottom panel) Alternatively, a diverse and largely anaerobic bacterial population that is part of the BV-CVM may help facilitate *T. vaginalis* colonization. (**b**) Competitive and synergistic interactions of *T. vaginalis* with microbial communities of the CVM. Protective lactobacilli and *T. vaginalis* are competitors in the vagina. The parasite inhibits the growth of lactobacilli and the bacteria inhibit *T. vaginalis* adhesion to the epithelium. On the other hand, a dysbiotic BV-CVM (anaerobes) promotes *T. vaginalis* adhesion to pathogenic biofilm and host cells. BV bacteria are pathobionts of *T. vaginalis*; enhancing host cell and epithelial damage. Additionally, *T. vaginalis* and BV-CVM impact the host immune response synergistically. Killing of *T. vaginalis* by host immune defense (e.g. trogocytosis) or by the action of the 5-nitromidazole drug is likely to release pathogenic *M. hominis*; which can invade vaginal cells. Although the drug might eliminate the parasite, it does not help restore microbiome disturbances. Figure A created with BioRender.com**.** Image B was reprinted from Trends in Parasitology, de Aquino et al. *Trichomonas vaginalis*, Parasite of the Month, Copyright (2020) with permission from Elsevier

5.2.4 The Interplay of Vaginal Bacteria, *T. vaginalis* Virulence, and Host Response

The interactions of *T. vaginalis* with the microbiota are certainly influential to disease and have important consequences to women's sexual health. Because the vaginal microbiome composition appears to be largely human specific (as detailed in Sect. 5.2.1) (Vrbanac et al. 2018; Yildirim et al. 2014b), replicating a *T. vaginalis* infection together with its associated microbiome will be highly challenging, if possible at all, with any animal model. However, investigating how *T. vaginalis* modulates endogenous CVMs in different animals may help reveal common effects exerted by *T. vaginalis* on specific bacterial species.

Currently, to investigate the influence of the CVM on *T. vaginalis* virulence and host responses, researchers have applied tissue culture models of microbial colonization and infection. These models use immortalized ectocervical or vaginal cells that maintain the expression of immune and physiological markers from their tissues of origin (Fichorova et al. 1997). Ex-vivo models of the human cervicovaginal mucosa, e.g. the commercially available EpiVaginal cultures (MatTek Corporation),

represent a further step towards replicating the physiological complexity of this tissue. These cultures are established from primary epithelial cells conditioned to form highly differentiated, polarized and multi-layered cervicovaginal tissue resembling the structure and physiology of the native tissue. In addition to being supplemented with immune cells, human microbiomes can be transplanted to, or defined microbial communities can be established on these 3D multilayer cultures [reviewed in Herbst-Kralovetz et al. 2016]. Tissue damage and immunotoxicity in response to commensals and pathogens can be evaluated on these organotypic systems; which are also quite valuable to assess biocompatibility of microbicides (Trifonova et al. 2006).

Although no artificial system can recapitulate the entire physiological complexity of the cervicovaginal mucosa, in vitro and ex vivo culture systems have been very useful to establish models for microbial colonization of the vagina (Fichorova et al. 2011) with particular emphasis on BV [reviewed in Herbst-Kralovetz et al. 2016]. Importantly, the use of culture systems is now providing evidence that the correlation between trichomoniasis and CST-IV is not merely coincidental. The interaction of *T. vaginalis* with the vaginal commensals has profound effects on parasite virulence and host responses (Fichorova et al. 2013; Hinderfeld et al. 2019; Hinderfeld and Simoes-Barbosa 2019; Phukan et al. 2013, 2018). Protective lactobacilli, particularly the most stable vaginal species *L. gasseri*, are very inhibitory to the adhesion of *T. vaginalis* to human ectocervical cells (Ect-1) (Phukan et al. 2013), compared to other bacteria. *L. gasseri* can preclude parasites from binding to, or displace already bound parasites from host cells during short incubation periods (30–60 min), even against very cytoadherent strains of *T. vaginalis*. This inhibitory phenotype depends on the physical contact between bacteria and parasite, with participation of bacterial surface proteins that promote aggregation. A specific *L. gasseri* aggregation-promoting factor was identified to participate in this inhibition (Phukan et al. 2018). *T. vaginalis*, on the other hand, can reduce the colonization of Ect-1 by lactobacilli but not BV-associated bacteria after long incubation periods (>24 h) (Fichorova et al. 2013). Although there is much more to be understood, these findings support the hypothesis that *T. vaginalis* and protective lactobacilli are natural competitors in the vagina.

Contrary to protective lactobacilli, BV bacteria were found to enhance the adhesion of *T. vaginalis* to Ect-1 cells very significantly (Hinderfeld and Simoes-Barbosa 2019) (Fig. 6b). *T. vaginalis* G3 (the genome strain) is a very lowly cytoadherent strain (Brooks et al. 2013; Lustig et al. 2013). However, in the presence of BV bacteria, G3 adheres to Ect-1 as strongly as the highly cytoadherent *T. vaginalis* strain B7RC2. In addition, BV bacteria were found to delay the binding of *T. vaginalis* to mucins, the major component of the host-protective mucus barrier. A notorious activity of BV bacteria is mucin deglycosylation, particularly by removal of sialic acid via sialidases (Howe et al. 1999; Lewis et al. 2013; Wiggins et al. 2001). Sialic acid is a major moiety responsible for the rheological properties of mucins (Ridley and Thornton 2018; Wiggins et al. 2001), thus BV bacteria can make mucous less viscous or rigid. *T. vaginalis* is capable of proteolytic degradation of mucins (Lehker and Sweeney 1999); however *T. vaginalis* sialidase is enzymatically

incapable of removing alpha 2-6 linked sialic acid from mucins (Padilla-Vaca and Anaya-Velazquez 1997). Therefore, BV bacteria and *T. vaginalis* may have complementary activities, acting cooperatively on the modification of host mucins at the vaginal mucosal interface. Interestingly, prokaryotic and eukaryotic microbes that live in association with animal host mucosa were found to have a common set of proteins carrying a zinc-metallopeptidase-like motif (M60-like/PF13402 domain) with an extracellular signature and, in some cases, glycan-binding domains (Nakjang et al. 2012). *T. vaginalis* was found to have 25 genes encoding proteins with the PF13402 domain and a bacterial enzyme containing this domain was shown to cause proteolytic degradation of mucins in vitro (Nakjang et al. 2012). Thus, *T. vaginalis* has a large uncharacterized repertoire of putative mucinases. It will be important to understand how BV and *T. vaginalis* act together on the modification of the host mucins, as this is the "first line of defense" serving as the outermost physical barrier of the mucosa.

Although experimental support is still lacking, cooperative modification and degradation of mucins between BV bacteria and *T. vaginalis* could provide advantages to both microorganisms. This cooperative activity could support microbial nutrition from alternative sugars and amino acids released from the degraded mucin. Sialidases from BV bacteria may be important to initiate the process of breaching this protective mucus layer. This is because BV sialidases are likely to soften the gel layer structure, potentially granting *T. vaginalis* access to bind and proteolytically degrade mucins. Together, these activities would help both bacteria and parasite break through the mucus and reach the underlying epithelium. Mucinase and sialidase activities of BV bacteria were found to be implicated in the pathogenesis of preterm labor (Howe et al. 1999; McGregor et al. 1994). A CST-IV colonized mucus was found to facilitate the mobility of HIV virions, as compared to a *L. crispatus* (CST-I) colonized mucus (Hoang et al. 2020). Hence, the cooperative activities of BV bacteria and *T. vaginalis* on mucins might also be a contributor to increased HIV infection susceptibility and preterm labor observed during trichomoniasis (Kissinger 2015).

It is also possible that BV bacteria could further enhance *T. vaginalis* adherence to the cervicovaginal epithelium in vivo, due to the biofilm forming-properties of BV bacteria. This polymicrobial biofilm, initiated by *G. vaginalis*, is a major clinical feature of BV that makes treatment more difficult, and is implicated in higher rates of recurrence (Machado and Cerca 2015; Swidsinski et al. 2005). A minimal in vitro biofilm produced by *G. vaginalis* was shown to be an excellent substrate for *T. vaginalis* adhesion (Hinderfeld and Simoes-Barbosa 2019) (Fig. 6b). It will be important to understand whether BV-derived biofilms provide a niche for metabolic exchanges between bacteria and *T. vaginalis*, and additionally contribute to drug tolerance, as has been observed between BV bacteria themselves (Machado and Cerca 2015). Additionally, this interaction within the biofilm may be advantageous for both bacteria and protozoa. As a parallel example, *Giardia lamblia* (an extracellular protozoan parasite of the human gut mucosa) is capable of modifying ex vivo gut biofilms promoting the release of specific bacteria (Beatty et al. 2017). This feature might explain dysbiosis and post-infectious complications

associated with giardiasis (Allain et al. 2017). If *T. vaginalis* can similarly modify bacterial biofilms, this processs may help disperse and seed specific bacteria to different areas of the ectocervix and vagina, contributing to dysbiosis and potentially driving changes of the microbiome to a CST-IV.

T. vaginalis evolution alongside the CVM has likely also given opportunities for genetic exchange. Interestingly, one family of genes in *T. vaginalis*, the NlpC/P60 genes, were recently shown to be inherited by horizontal gene transfer (HGT) from bacteria (Pinheiro et al. 2018). NlpC/P60 peptidases were originally identified as D, L-endopeptidases of peptidoglycan (PG), the major polymer of bacterial cell walls. Despite phylogenetic signal being insufficient to identify the bacterial ancestor, researchers predicted two possible events of HGT followed by gene duplications that led to a family of nine NlpC/P60 genes in the *T. vaginalis* genome (Pinheiro et al. 2018). The characterization of two of these *T. vaginalis* NlpC/P60 enzymes (TvNlpC/P60) have shown that they are bonafide PG hydrolases (Pinheiro et al. 2018) with a classical papain-like fold and catalytic triad, in addition to two bacterial SH3 domains giving a unique tri-dimensional arrangement (Pinheiro et al. 2018). These enzymes are fully capable of cleaving the model PG of *Escherichia coli*, between D-isoGlu and m-DAP residues and with preference for mature PG (Pinheiro et al. 2018). The TvNlpC/P60 enzymes were detectable on the surface of the parasite, and also secreted (Pinheiro et al. 2018). Their presence was also identified in the *T. vaginalis* secretome (Stafkova et al. 2018). Interestingly, TvNlpC/P60 expression was upregulated upon *T. vaginalis* contact with bacteria, and exogenous expression of the enzymes in *T. vaginalis* led to a dramatic capability of controlling the *E. coli* population in mixed cultures (Pinheiro et al. 2018). Therefore, these genes, once 'stolen' from bacteria, are apparently used by the parasite against them. Although this was the first time that a specific factor from *T. vaginalis* was shown to contribute directly towards controlling bacteria populations, at least in vitro, many questions remain. It is unclear if other factors (human- or parasite-derived) may support the potential role of TvNlpC/P60 in controlling bacterial populations. For instance, vaginal secretions are known to have the most abundant levels of lysozyme of all mucosal sites (Bard et al. 2003). It is also unclear how these *T. vaginalis* enzymes reach the PG of bacterial cells and whether there is bacterial target specificity. Finally, these enzymes may possibly provide other advantages to the parasite such as nutrition and/or immune modulation, as PG fragments are known to be ligands of pattern recognition receptors (Chu and Mazmanian 2013; Wolf and Underhill 2018).

Finally, evidence supports that BV bacteria and *T. vaginalis* synergistically impact the host immune response to *T. vaginalis* (RN Fichorova et al. 2013). Some BV bacteria (particularly *G. vaginalis* and *A. vaginae*) act in concert with *T. vaginalis* to amplify pro-inflammatory responses, promoting secretion of chemokines such as IL-8 and RANTES; effects not seen with lactobacilli controls (RN Fichorova et al. 2013). Increases in IL-8 are predicted to attract more neutrophils to the infection site, which although they are players killing *T. vaginalis*, also highly promote inflammation. Increases in RANTES, which can recruit CCR5+ leukocytes to the infection site, are linked to increased HIV susceptibility, as

discussed in Sect. 4.3 (Kissinger 2015). Furthermore, *G. vaginalis* and *A. vaginae* were found to significantly enhance *T.vaginalis*-mediated disruption of paracellular permeability, in a cervicovaginal epithelial model (Hinderfeld et al. 2019). The microbial-driven increase in permeability was not due to cytotoxicity, but was accompanied by dysregulation of the tight junctions of the epithelium. The major component occludin was virtually excluded from the junctional complex, possibly due to high levels of phosphatase activity, as detected in this study (Hinderfeld et al. 2019). Increase of paracellular permeability of the ectocervical epithelium allows bacteria, viruses and toxins to reach the underlying tissue. Dysfunction of the cervicovaginal epithelium induces cervical remodelling, the ripening process of the cervix necessary for delivery of the foetus (Yellon 2017). Once enhanced by BV bacteria and *T. vaginalis* synergistically, early onset of cervical remodelling in pregnant women with trichomoniasis might explain the association of these infections with preterm birth (Kissinger 2015).

As with many other parasites, *T. vaginalis* research has traditionally focused on host and parasite in isolation. As detailed above, recent progress has been achieved towards understanding trichomoniasis in the context of the triad host: parasite: microbiota. In the near future, it will be important to decipher the mechanisms and the genetic basis of microbial competitions and trade-offs that either support vaginal eubiosis or drive dysbiosis during *T. vaginalis* infection. Once mechanisms are better understood, a new avenue for interventions against trichomoniasis - that consider restoring the normal function of the vaginal biome - might be envisaged.

6 Part 6: Treatment and Vaccination Prospects

T. vaginalis has been historically diagnosed through wet mount (observations of trichomonads microscopically), but several higher sensitivity and more rapid PCR tests are now also available, which can detect *T. vaginalis* in vaginal/cervical swabs, and urine (Van Gerwen and Muzny 2019). However, many cases of *T. vaginalis* go undiagnosed as testing for it is largely symptom-driven and due to its prevalence in resource-poor settings. Knowledge of *T. vaginalis* biology and metabolism has enabled the use of good treatment options that can clear most symptomatic infections. Still, alternative therapies that consider the complexities in the host-pathogen interface discussed above, including those that consider the “microbiomes” of both host and parasite, may help to inform novel therapeutic options that may be useful during pregnancy, or in the face of rising drug resistence. A protective vaccine is also a very attractive prospect for this highly prevalent human infection, which disproportionately affects those with limited access to healthcare.

6.1 5′ Nitroimidazole Drugs

If diagnosed, most infections can be cleared with treatment by the 5-nitroimidazole drugs metronidazole (Mz) and tinidazole (Van Gerwen and Muzny 2019). Metronidazole is the standard first line of treatment, and tinidazole is used less commonly due to its higher cost (Muzny et al. 2020). The 5-nitroimidazoles are prodrugs, that need to be activated inside *T. vaginalis* after passive entry into the parasite. Specifically, these classes of drugs are activated inside the hydrogenosome via redox enzymes and/or flavin reductases that reduce the prodrugs (Hrdy et al. 2005). Thus, the parasite's anaerobic metabolism confers susceptibility (Leitsch 2016). Resistance to Mz is currently estimated to be as high as 5% in the US (Leitsch et al. 2012). Alarmingly, in Papau New Guinea, where *T. vaginalis* is endemic (prevalence of 46% and 33% have been reported from different regions), drug resistance was found to be 17.4% (Upcroft et al. 2009). Resistance to tinidazole, often used against Mz-resistant cases, is also emerging, and present in some Mz-resistant strains (Leitsch et al. 2012). Despite the increasing levels of Mz resistance, the *T. vaginalis* mechanisms that have evolved to resist Mz are currently under-characterized. Bradic and colleagues recently identified 72 Single-Nucleotide Polymorphisms (SNPs) associated with moderate or high Mz resistance; proposing them as a panel of biomarkers that can be used to advance personalized treatment for trichomoniasis (Bradic et al. 2017). Transcriptomic analysis of Mz-resistant strains further provided a global view of molecular pathways involved in Mz resistance, highlighting a differential expression of genes that could be involved in drug activation, drug efflux, and detoxification (Bradic et al. 2017). However, the identification of specific targets of Mz would be useful in developing alternative drugs to target these essential proteins.

6.2 Alternative Treatments

Secindazole, another 5-nitroimidazole that is used against the protozoan *Dientamoeba fragilis,* shows 96% efficacy against Mz-resistant *T. vaginalis* strains tested, indicating that this could be efficacious against hard-to-treat infections (Ghosh et al. 2018). Since the *T. vaginalis* and mammalian proteasomes are divergent, the 20S proteasome was also recently shown to be a promising new drug target (O'Donoghue et al. 2019). Other proposed treatments include boric acid suppository (Van Gerwen and Muzny 2019), ceragenins, and topical mimics of antimicrobial peptides which can kill Mz-resistant strains in vitro (Polat et al. 2011), as well as natural products such as the *Manilkara Rufula* plant extract, which killed *T. vaginalis* but not host cells in vitro (de Brum Vieira et al. 2017a, b). As recently reviewed, there is a phletora of natural compounds with potential to lead to alternative treatments for trichomoniasis (Friedman et al. 2020). Many of theses natural products have been shown to kill *T. vaginalis* (and other trichomonads) or display

some form of anti-trichomonad activity, whether in vitro, in animals, or in humans, and have sometimes been shown to enhace the efficacy of Mz. It is undeniable that new more efficacious treatment strategies against *T. vaginalis* are needed.

6.3 Vaccines

The high prevalence of *T. vaginalis* and the increasing reports of strains refractory to treatment make a preventative vaccine an attractive goal. However, several confounding factors currently complicate vaccine development. Firstly, a more thorough understanding of the mechanisms of effective immune clearance, and memory to the parasite is necessary to define the best immunogens and adjuvants. Establishing a mouse model of infection (or any other animal) would aid in better understanding of in vivo pathogenesis, and for testing immunogens and adjuvants. At this stage, the immune response to *T. vaginalis* remains under-characterized, and a mouse model that is able to retain adequate titers of infection to allow in vivo studies of pathogenesis and immune responses remains challenging, possibly due to differences in murine and human vaginal pH, microbial content, and availability of appropriate adhesin receptors. The most successful murine models of *T. vaginalis* have used estrogen and dexamethasone pre-treatment in order to promote *T. vaginalis* colonization (Cobo et al. 2011), which are both immunosuppressive, making these models problematic for studies of immune function. Furthermore, the required inoculum to establish any infection is extremely high (one million parasites), and only 20–70% of mice inoculated in these studies become infected, indicating a failure of most parasites inoculated to survive and colonize the mouse vagina, or rapid clearance of parasites (Cobo et al. 2011; Smith and Garber 2015). Despite these challenges, mice vaccinated with a whole cell killed *T. vaginalis* vaccine containing alum adjuvant were shown to be infected by vaginal inoculation at lower rates than unvaccinated controls, indicating promise for continued *T. vaginalis* vaccine development efforts (Smith and Garber 2015).

A vaccine does exist against a related vaginal trichomonad, *Tritrichomonas foetus*, which infects cattle (Palomares et al. 2017). However, while this vaccine, called TrichGuard can reduce the incidence of spontaneous abortion and infertility on endemic farms, it does not confer robust immunological memory, as it requires re-administration and does not completely prevent infection (Edmondson et al. 2017). TrichGuard uses whole-cell killed trichomonad and oil adjuvant (Palomares et al. 2017), and was shown to induce vaginal and uterine antibodies against the major surface antigen of the parasite in its native conformation (Palomares et al. 2017), indicating that whole- killed or purified surface antigen of *T. vaginalis* might be good immunogens to test in a *T. vaginalis* vaccine. Furthermore, in a mouse model utilizing intraperitoneal injection of *T. vaginalis* and death of mice as a marker of *T. vaginalis* pathogenesis, immunization with recombinant *T. vaginalis* alpha-actinin conferred enhanced survival and higher cytokine levels (Xie et al. 2017). It is not clear whether these findings are applicable to vaginal infection or would provide

protection against infection acquisition. In addition to prophylactic vaccines, it may also be interesting to think about therapeutic vaccines that could confer enhanced control against adverse consequences during pregnancy, since Mz is not currently efficacious in decreasing pre-term births for *T. vaginalis*-infected pregnant women (Klebanoff et al. 2001). Overall, more research into alternative therapies and vaccination strategies against this highly prevalent parasite is needed.

7 Part 7: Conclusions and Perspectives

As described previously, *Trichomonas vaginalis* has unique cellular and molecular features, engaging with myriad host and microbial cells in different ways. In addition to host immunity and parasite evasion, endosymbionts of *T. vaginalis* and the accompanying indigenous microbiome contribute to pathogenesis of trichomoniasis (Fig. 6b). The discovery of extracellular vesicles that *T. vaginalis* produces, and the observation of trogocytosis in host-parasite interactions also suggest new exciting modes of cell interaction and communication that blur the boundaries of cells and their membranes. While the publication of the *T. vaginalis* genome in 2007 and subsequent *omics* research have aided these investigations considerably, the poor annotation of the published genome and the lack of genome sequences for more strains of the parasite have hindered additional progress. Clinical isolates of the parasite vary considerably in their in vitro behaviors towards host cells, and differing inherent virulence among strains very likely contributes to the varying clinical outcomes observed. We therefore advocate for the development of more datasets on different clinical isolates, including sequencing of genomes and transcriptomes. Additional functional studies comparing different strains would also bolster our understanding of the contribution of strain type to outcomes, and a greater attention to strain identity is important when comparing results from different studies. On this note, functional characterization of virulence factors and other molecular players will be greatly bolstered by improved genetic tools, such as the CRISPR/Cas9 system, recently adapted for use in *T. vaginalis* (Janssen et al. 2018).

A substantial hurdle for studying *T. vaginalis* pathogenesis is the lack of a suitable animal model whereby infections can be naturally established and maintained chronically. Such an animal model would allow scientists to test hypotheses or translate their findings in the laboratory to a real infection situation, teasing out key molecular players that contribute to pathogenesis in vivo. Researchers would be able to study the efficacy of innate and adative immune responses as well as immune-evasive behaviors of the parasite in vivo. The contribution of microbial interactions (symbionts and microbiome) to the pathogenesis of trichomoniasis could then be characterized in the context of the host response in a more realistic scenario. Undoubtedly, these animal models would allow more translatable research that could aid the development of immunotherapy interventions or vaccine design.

However, popular murine models for infection are likely to pose significant limitations for studying certain aspects of trichomoniasis. Firstly, humans and

rodents do not share all immune receptors and effector molecules. For example, IL-8, the predominant cytokine released in *T. vaginalis*-stimulated cells and the major chemokine thought to mobilize neutrophils against *T. vaginalis* in the human system, does not exist in mice, and polarizing cytokine requirements for Th17 responses, which are suspected to be important during trichomoniasis, differ slightly in mice versus humans (de Jong et al. 2010). Secondly, the physiology of women's reproductive tract comprises a mutualistic host-microbiome relationship (Yildirim et al. 2014a; Younes et al. 2018) that is unique across the animal kingdom. Although a 'humanized' immune system in mice is a viable option (Li and Di Santo 2019; Walsh et al. 2017) and has been utilized with other parasites successfully (Tyagi et al. 2018), humanizing the CVM of mice stably may not be practical or possible. Despite this, encouraging results have been achieved with BV models in mice. Recently, transient cervicovaginal colonization of mice with BV-bacteria was able to recapitulate some typical features of BV (Gilbert et al. 2019). In pregnant mice, this microbial colonization was accompanied by the expression of molecular, immune and cellular markers, and physiological changes typical of cervical remodeling (Sierra et al. 2018). When triggered prematurely, cervical remodelling will lead to preterm birth. Although very speculative, a partial or transient reconstruction of the human CVM in mice may possibly facilitate *T. vaginalis* infection.

The investigation of trichomoniasis in males is on the horizon. A recent study by Jang et al. has developed a rat model of *T. vaginalis* infecton using a combination of multiple *T. vaginalis* infections and *T. vaginalis* excretory-secretory products (Jang et al. 2019). This study found increased levels of CCL2 and mast cell infiltration in the prostate of *T. vaginalis*-infected rats. Future studies with this model will aid dissection of the inflammatory steps that promote *T. vaginalis*-induced prostatitis.

T. vaginalis treatment has been solely dependent on 5′ nitromidazoles ever since the development of metronidazole in 1959 (Cosar and Julou 1959). While *T. vaginalis'* anaerobic metabolism makes this drug quite efficient and specific, the rise in metronidazole resistance has signalled the need to develop novel and alternative treatment approaches for this highly prevalent human infection. We envisage that new interventions should be guided by a holistic understanding of the parasite cellular and molecular biology and its interactions with host and microbiome. A proper understanding of this disease requires attention to the biology of *T. vaginalis* and microbial ecology. It is important to identify key players in parasite metabolism, growth, and virulence; but it is also critical to understand its interaction with other cells including from the host, endosymbionts and commensals. Therefore, further studies on the the interactions with the endosymbionts (*M. hominis*, *M. girerdii*, and *Trichomonasvirus*) and microbiome are needed.

T. vaginalis frequently carries pathogenic *Mycoplasma.* In addition, trichomoniasis is often accompanied by a dysbiotic microbiome typical of BV. Metronidazole therapy does not eliminate *Mycoplasma* and the majority of treated women will experience either recurrent BV or an abnormal microbiome (Bradshaw et al. 2006). Thus, novel interventions should aim to eliminate *T. vaginalis* and *Mycoplasma* concomitantly in order to restore the healthy functional status of the immune- and microbial biomes. If this intervention strategy can be developed in the future, it will

boost protection to re-infections and mitigate co-morbidities that are clinically relevant and commonly associated with trichomoniasis.

References

Abe J, Morrell C (2016) Pyroptosis as a regulated form of necrosis: PI+/annexin V-/high caspase 1/low caspase 9 activity in cells = pyroptosis? Circ Res 118(10):1457–1460. https://doi.org/10.1161/circresaha.116.308699

Abedin M, King N (2010) Diverse evolutionary paths to cell adhesion. Trends Cell Biol 20 (12):734–742. https://doi.org/10.1016/j.tcb.2010.08.002

Abels ER, Breakefield XO (2016) Introduction to extracellular vesicles: biogenesis, RNA cargo selection, content, release, and uptake. Cell Mol Neurobiol 36(3):301–312. https://doi.org/10.1007/s10571-016-0366-z

Alderete JF (1999) Iron modulates phenotypic variation and phosphorylation of P270 in double-stranded RNA virus-infected Trichomonas vaginalis. Infect Immun 67(8):4298–4302

Alderete JF, Garza GE (1985) Specific nature of Trichomonas vaginalis parasitism of host cell surfaces. Infect Immun 50(3):701–708

Alderete JF, Pearlman E (1984) Pathogenic Trichomonas vaginalis cytotoxicity to cell culture monolayers. Br J Vener Dis 60(2):99–105. https://doi.org/10.1136/sti.60.2.99

Allain T, Amat CB, Motta JP, Manko A, Buret AG (2017) Interactions of Giardia sp. with the intestinal barrier: epithelium, mucus, and microbiota. Tissue Barriers 5(1):e1274354. https://doi.org/10.1080/21688370.2016.1274354

Alvarez-Sanchez ME, Avila-Gonzalez L, Becerril-Garcia C, Fattel-Facenda LV, Ortega-Lopez J, Arroyo R (2000) A novel cysteine proteinase (CP65) of Trichomonas vaginalis involved in cytotoxicity. Microb Pathog 28(4):193–202. https://doi.org/10.1006/mpat.1999.0336

Alvarez-Sanchez ME, Solano-Gonzalez E, Yanez-Gomez C, Arroyo R (2007) Negative iron regulation of the CP65 cysteine proteinase cytotoxicity in Trichomonas vaginalis. Microbes Infect 9(14-15):1597–1605. https://doi.org/10.1016/j.micinf.2007.09.011

Alvarez-Sanchez ME, Carvajal-Gamez BI, Solano-Gonzalez E, Martinez-Benitez M, Garcia AF, Alderete JF, Arroyo R (2008) Polyamine depletion down-regulates expression of the Trichomonas vaginalis cytotoxic CP65, a 65-kDa cysteine proteinase involved in cellular damage. Int J Biochem Cell Biol 40(11):2442–2451. https://doi.org/10.1016/j.biocel.2008.04.023

Amabebe E, Anumba DOC (2018) The vaginal microenvironment: the physiologic role of lactobacilli. Front Med (Lausanne) 5:181. https://doi.org/10.3389/fmed.2018.00181

Anahtar MN, Gootenberg DB, Mitchell CM, Kwon DS (2018) Cervicovaginal microbiota and reproductive health: the virtue of simplicity. Cell Host Microbe 23(2):159–168. https://doi.org/10.1016/j.chom.2018.01.013

Andreu Z, Yanez-Mo M (2014) Tetraspanins in extracellular vesicle formation and function. Front Immunol 5:442. https://doi.org/10.3389/fimmu.2014.00442

Arroyo R, Alderete JF (1989) Trichomonas vaginalis surface proteinase activity is necessary for parasite adherence to epithelial cells. Infect Immun 57(10):2991–2997

Arroyo R, Gonzalez-Robles A, Martinez-Palomo A, Alderete JF (1993) Signalling of Trichomonas vaginalis for amoeboid transformation and adhesion synthesis follows cytoadherence. Mol Microbiol 7(2):299–309. https://doi.org/10.1111/j.1365-2958.1993.tb01121.x

Arroyo R, Cardenas-Guerra RE, Figueroa-Angulo EE, Puente-Rivera J, Zamudio-Prieto O, Ortega-Lopez J (2015) Trichomonas vaginalis cysteine proteinases: iron response in gene expression and proteolytic activity. Biomed Res Int 2015:946787. https://doi.org/10.1155/2015/946787

Aurrecoechea C, Brestelli J, Brunk BP, Carlton JM, Dommer J, Fischer S, Gajria B, Gao X, Gingle A, Grant G, Harb OS, Heiges M, Innamorato F, Iodice J, Kissinger JC, Kraemer E, Li W, Miller JA, Morrison HG, Nayak V, Pennington C, Pinney DF, Roos DS, Ross C,

Stoeckert CJ, Sullivan S, Treatman C, Wang HM (2009) GiardiaDB and TrichDB: integrated genomic resources for the eukaryotic protist pathogens Giardia lamblia and Trichomonas vaginalis. Nucleic Acids Res 37:D526–D530. https://doi.org/10.1093/Nar/Gkn631

Balczun C, Scheid PL (2017) Free-living amoebae as hosts for and vectors of intracellular microorganisms with public health significance. Viruses 9(4):65. https://doi.org/10.3390/v9040065

Bao Y, Al KF, Chanyi RM, Whiteside S, Dewar M, Razvi H, Reid G, Burton JP (2017) Questions and challenges associated with studying the microbiome of the urinary tract. Ann Transl Med 5 (2):33. https://doi.org/10.21037/atm.2016.12.14

Bard E, Laibe S, Bettinger D, Riethmuller D, Biichle S, Seilles E, Meillet D (2003) New sensitive method for the measurement of lysozyme and lactoferrin for the assessment of innate mucosal immunity. part I: time-resolved immunofluorometric assay in serum and mucosal secretions. Clin Chem Lab Med 41(2):127–133. https://doi.org/10.1515/CCLM.2003.021

Bastida-Corcuera FD, Okumura CY, Colocoussi A, Johnson PJ (2005) Trichomonas vaginalis lipophosphoglycan mutants have reduced adherence and cytotoxicity to human ectocervical cells. Eukaryot Cell 4(11):1951–1958. https://doi.org/10.1128/EC.4.11.1951-1958.2005

Bastida-Corcuera FD, Singh BN, Gray GC, Stamper PD, Davuluri M, Schlangen K, Corbeil RR, Corbeil LB (2013) Antibodies to Trichomonas vaginalis surface glycolipid. Sex Transm Infect 89(6):467–472. https://doi.org/10.1136/sextrans-2012-051013

Bauer MA, Kainz K, Carmona-Gutierrez D, Madeo F (2018) Microbial wars: Competition in ecological niches and within the microbiome. *Microb. Cell 5*(5):215–219. https://doi.org/10.15698/mic2018.05.628

Beatty JK, Akierman SV, Motta JP, Muise S, Workentine ML, Harrison JJ, Bhargava A, Beck PL, Rioux KP, McKnight GW, Wallace JL, Buret AG (2017) Giardia duodenalis induces pathogenic dysbiosis of human intestinal microbiota biofilms. Int J Parasitol 47(6):311–326. https://doi.org/10.1016/j.ijpara.2016.11.010

Benchimol M (2004) Trichomonads under microscopy. Microsc Microanal 10(5):528–550. https://doi.org/10.1017/s1431927604040905

Benchimol M (2010) The mastigont system in trichomonads. In: de Souza W (ed) Structures and organelles in pathogenic protists. Springer, Berlin, pp 1–26

Benchimol M, De Souza W (1990) Freeze-fracture study of Trichomonas vaginalis. Mem Inst Oswaldo Cruz 85(4):419–428. https://doi.org/10.1590/s0074-02761990000400006

Benchimol M, Johnson PJ, de Souza W (1996) Morphogenesis of the hydrogenosome: an ultrastructural study. Biol Cell 87(3):197–205

Benchimol M, Ribeiro KC, Mariante RM, Alderete JF (2001) Structure and division of the Golgi complex in Trichomonas vaginalis and Tritrichomonas foetus. Eur J Cell Biol 80(9):593–607. https://doi.org/10.1078/0171-9335-00191

Benchimol M, de Andrade Rosa I, da Silva Fontes R, Burla Dias AJ (2008) Trichomonas adhere and phagocytose sperm cells: adhesion seems to be a prominent stage during interaction. Parasitol Res 102(4):597–604. https://doi.org/10.1007/s00436-007-0793-3

Berndorff D, Gessner R, Kreft B, Schnoy N, Lajous-Petter AM, Loch N, Reutter W, Hortsch M, Tauber R (1994) Liver-intestine cadherin: molecular cloning and characterization of a novel Ca (2+)-dependent cell adhesion molecule expressed in liver and intestine. J Cell Biol 125 (6):1353–1369

Boris S, Barbes C (2000) Role played by lactobacilli in controlling the population of vaginal pathogens. Microbes Infect 2(5):543–546. https://doi.org/10.1016/s1286-4579(00)00313-0

Bradic M, Carlton JM (2018) Does the common sexually transmitted parasite Trichomonas vaginalis have sex? PLoS Pathog 14(3):e1006831. https://doi.org/10.1371/journal.ppat.1006831

Bradic M, Warring SD, Tooley GE, Scheid P, Secor WE, Land KM, Huang PJ, Chen TW, Lee CC, Tang P, Sullivan SA, Carlton JM (2017) Genetic indicators of drug resistance in the highly repetitive genome of Trichomonas vaginalis. Genome Biol Evol 9(6):1658–1672. https://doi.org/10.1093/gbe/evx110

Bradshaw CS, Morton AN, Hocking J, Garland SM, Morris MB, Moss LM, Horvath LB, Kuzevska I, Fairley CK (2006) High recurrence rates of bacterial vaginosis over the course of 12 months after oral metronidazole therapy and factors associated with recurrence. J Infect Dis 193(11):1478–1486. https://doi.org/10.1086/503780

Bricheux G, Coffe G, Brugerolle G (2007) Identification of a new protein in the centrosome-like "atractophore" of Trichomonas vaginalis. Mol Biochem Parasitol 153(2):133–140. https://doi.org/10.1016/j.molbiopara.2007.02.011

Brooks AE, Parsamand T, Kelly RW, Simoes-Barbosa A (2013) An improved quantitative method to assess adhesive properties of Trichomonas vaginalis to host vaginal ectocervical cells using flow cytometry. J Microbiol Methods 92(1):73–78. https://doi.org/10.1016/j.mimet.2012.10.011

Brotman RM, Bradford LL, Conrad M, Gajer P, Ault K, Peralta L, Forney LJ, Carlton JM, Abdo Z, Ravel J (2012) Association between Trichomonas vaginalis and vaginal bacterial community composition among reproductive-age women. Sex Transm Dis 39(10):807–812. https://doi.org/10.1097/OLQ.0b013e3182631c79

Cacciotto C, Dessi D, Cubeddu T, Cocco AR, Pisano A, Tore G, Fiori PL, Rappelli P, Pittau M, Alberti A (2019) MHO_0730 as a surface-exposed calcium-dependent nuclease of mycoplasma hominis promoting neutrophil extracellular trap formation and escape. J Infect Dis 220 (12):1999–2008. https://doi.org/10.1093/infdis/jiz406

Cardenas-Guerra RE, Arroyo R, Rosa de Andrade I, Benchimol M, Ortega-Lopez J (2013) The iron-induced cysteine proteinase TvCP4 plays a key role in Trichomonas vaginalis haemolysis. Microbes Infect 15(13):958–968. https://doi.org/10.1016/j.micinf.2013.09.002

Carlton JM, Hirt RP, Silva JC, Delcher AL, Schatz M, Zhao Q, Wortman JR, Bidwell SL, Alsmark UC, Besteiro S, Sicheritz-Ponten T, Noel CJ, Dacks JB, Foster PG, Simillion C, Van de Peer Y, Miranda-Saavedra D, Barton GJ, Westrop GD, Muller S, Dessi D, Fiori PL, Ren Q, Paulsen I, Zhang H, Bastida-Corcuera FD, Simoes-Barbosa A, Brown MT, Hayes RD, Mukherjee M, Okumura CY, Schneider R, Smith AJ, Vanacova S, Villalvazo M, Haas BJ, Pertea M, Feldblyum TV, Utterback TR, Shu CL, Osoegawa K, de Jong PJ, Hrdy I, Horvathova L, Zubacova Z, Dolezal P, Malik SB, Logsdon JM Jr, Henze K, Gupta A, Wang CC, Dunne RL, Upcroft JA, Upcroft P, White O, Salzberg SL, Tang P, Chiu CH, Lee YS, Embley TM, Coombs GH, Mottram JC, Tachezy J, Fraser-Liggett CM, Johnson PJ (2007) Draft genome sequence of the sexually transmitted pathogen Trichomonas vaginalis. Science 315(5809):207–212. https://doi.org/10.1126/science.1132894

Caruso S, Poon IKH (2018) Apoptotic cell-derived extracellular vesicles: more than just debris. Front Immunol 9:1486. https://doi.org/10.3389/fimmu.2018.01486

Cauci S, Culhane JF (2007) Modulation of vaginal immune response among pregnant women with bacterial vaginosis by Trichomonas vaginalis, Chlamydia trachomatis, Neisseria gonorrhoeae, and yeast. *Am J Obstet Gynecol 196*(2):133 e131–133 e137. https://doi.org/10.1016/j.ajog.2006.08.033

Chang JH, Ryang YS, Kim SK, Park JY (2004) Trichomonas vaginalis-induced apoptosis in RAW264.7 cells is regulated through Bcl-xL, but not Bcl-2. Parasite Immunol 26 (3):141–150. https://doi.org/10.1111/j.0141-9838.2004.00693.x

Chen Z, O'Shea JJ (2008) Th17 cells: a new fate for differentiating helper T cells. Immunol Res 41 (2):87–102. https://doi.org/10.1007/s12026-007-8014-9

Chen YP, Twu O, Johnson PJ (2018) Trichomonas vaginalis macrophage migration inhibitory factor mediates parasite survival during nutrient stress. *mBio 9*(3):00910-8. https://doi.org/10.1128/mBio.00910-18

Chen YP, Riestra AM, Rai AK, Johnson PJ (2019) A novel cadherin-like protein mediates adherence to and killing of host cells by the parasite Trichomonas vaginalis. *mBio 10*(3): e00720–e00719. https://doi.org/10.1128/mBio.00720-19

Cheng WH, Huang KY, Huang PJ, Hsu JH, Fang YK, Chiu CH, Tang P (2015) Nitric oxide maintains cell survival of Trichomonas vaginalis upon iron depletion. Parasit Vectors 8:393. https://doi.org/10.1186/s13071-015-1000-5

Cheon SH, Kim SR, Song HO, Ahn MH, Ryu JS (2013) The dimension of Trichomonas vaginalis as measured by scanning electron microscopy. Korean J Parasitol 51(2):243–246. https://doi.org/10.3347/kjp.2013.51.2.243

Choi DS, Kim DK, Kim YK, Gho YS (2015) Proteomics of extracellular vesicles: exosomes and ectosomes. Mass Spectrom Rev 34(4):474–490. https://doi.org/10.1002/mas.21420

Chu H, Mazmanian SK (2013) Innate immune recognition of the microbiota promotes host-microbial symbiosis. Nat Immunol 14(7):668–675. https://doi.org/10.1038/ni.2635

Chudnovskiy A, Mortha A, Kana V, Kennard A, Ramirez JD, Rahman A, Remark R, Mogno I, Ng R, Gnjatic S, Amir ED, Solovyov A, Greenbaum B, Clemente J, Faith J, Belkaid Y, Grigg ME, Merad M (2016) Host-Protozoan Interactions Protect from Mucosal Infections through Activation of the Inflammasome. Cell 167(2):444–456 e414. https://doi.org/10.1016/j.cell.2016.08.076

Cobo ER, Eckmann L, Corbeil LB (2011) Murine models of vaginal trichomonad infections. Am J Trop Med Hyg 85(4):667–673. https://doi.org/10.4269/ajtmh.2011.11-0123

Coceres VM, Alonso AM, Nievas YR, Midlej V, Frontera L, Benchimol M, Johnson PJ, de Miguel N (2015) The C-terminal tail of tetraspanin proteins regulates their intracellular distribution in the parasite Trichomonas vaginalis. Cell Microbiol 17(8):1217–1229. https://doi.org/10.1111/cmi.12431

Conrad M, Zubacova Z, Dunn LA, Upcroft J, Sullivan SA, Tachezy J, Carlton JM (2011) Microsatellite polymorphism in the sexually transmitted human pathogen Trichomonas vaginalis indicates a genetically diverse parasite. Mol Biochem Parasitol 175(1):30–38. https://doi.org/10.1016/j.molbiopara.2010.08.006

Conrad MD, Gorman AW, Schillinger JA, Fiori PL, Arroyo R, Malla N, Dubey ML, Gonzalez J, Blank S, Secor WE, Carlton JM (2012) Extensive genetic diversity, unique population structure and evidence of genetic exchange in the sexually transmitted parasite Trichomonas vaginalis. PLoS Negl Trop Dis 6(3):e1573. https://doi.org/10.1371/journal.pntd.0001573

Conrad MD, Kissinger P, Schmidt N, Martin DH, Carlton JM (2013) Genetic diversity of Trichomonas vaginalis reinfection in HIV-positive women. Sex Transm Infect 89(6):473–478. https://doi.org/10.1136/sextrans-2013-051053

Cosar C, Julou L (1959) The activity of 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (R. P. 8823) against experimental Trichomonas vaginalis infections. Ann Inst Pasteur (Paris) 96(2):238–241

Costello EK, Sun CL, Carlisle EM, Morowitz MJ, Banfield JF, Relman DA (2017) Candidatus Mycoplasma girerdii replicates, diversifies, and co-occurs with Trichomonas vaginalis in the oral cavity of a premature infant. Sci Rep 7(1):3764. https://doi.org/10.1038/s41598-017-03821-7

Coyte KZ, Schluter J, Foster KR (2015) The ecology of the microbiome: networks, competition, and stability. Science 350(6261):663–666. https://doi.org/10.1126/science.aad2602

Crosby RA, Charnigo RA, Weathers C, Caliendo AM, Shrier LA (2012) Condom effectiveness against non-viral sexually transmitted infections: a prospective study using electronic daily diaries. Sex Transm Infect 88(7):484–489. https://doi.org/10.1136/sextrans-2012-050618

Cuervo P, Cupolillo E, Britto C, Gonzalez LJ, FC, ES-F, Lopes, LC, Domont, GB, & De Jesus, JB. (2008) Differential soluble protein expression between Trichomonas vaginalis isolates exhibiting low and high virulence phenotypes. J Proteome 71(1):109–122. https://doi.org/10.1016/j.jprot.2008.01.010

Cui D, Han G, Shang Y, Mu L, Long Q, Du Y (2015) The effect of chronic prostatitis on zinc concentration of prostatic fluid and seminal plasma: a systematic review and meta-analysis. Curr Med Res Opin 31(9):1763–1769. https://doi.org/10.1185/03007995.2015.1072707

de Brum Vieira P, Feijo Silva NL, Silva DB, Lopes NP, da Silva AG, da Silva MV, Bastida J, Macedo AJ, Tasca T (2017a) The Caatinga endemic Manilkara rufula possesses remarkable activity against Trichomonas vaginalis and Tritrichomonas foetus. Exp Parasitol 173:18–28. https://doi.org/10.1016/j.exppara.2016.12.006

de Brum Vieira P, Silva NLF, Menezes CB, da Silva MV, Silva DB, Lopes NP, Macedo AJ, Bastida J, Tasca T (2017b) Trichomonicidal and parasite membrane damaging activity of

bidesmosic saponins from Manilkara rufula. PLoS One 12(11):e0188531. https://doi.org/10.1371/journal.pone.0188531

De Jesus JB, Cuervo P, Britto C, Saboia-Vahia L, Costa ES-FF, Borges-Veloso A, Barreiros Petropolis D, Cupolillo E, Barbosa Domont G (2009) Cysteine peptidase expression in Trichomonas vaginalis isolates displaying high- and low-virulence phenotypes. J Proteome Res 8 (3):1555–1564. https://doi.org/10.1021/pr8009066

de Jong E, Suddason T, Lord GM (2010) Translational mini-review series on Th17 cells: development of mouse and human T helper 17 cells. Clin Exp Immunol 159(2):148–158. https://doi.org/10.1111/j.1365-2249.2009.04041.x

de Miguel N, Lustig G, Twu O, Chattopadhyay A, Wohlschlegel JA, Johnson PJ (2010) Proteome analysis of the surface of Trichomonas vaginalis reveals novel proteins and strain-dependent differential expression. Mol Cell Proteomics 9(7):1554–1566. https://doi.org/10.1074/mcp.M000022-MCP201

de Miguel N, Riestra A, Johnson PJ (2012) Reversible association of tetraspanin with Trichomonas vaginalis flagella upon adherence to host cells. Cell Microbiol 14(12):1797–1807. https://doi.org/10.1111/cmi.12003

Dessi D, Delogu G, Emonte E, Catania MR, Fiori PL, Rappelli P (2005) Long-term survival and intracellular replication of Mycoplasma hominis in Trichomonas vaginalis cells: potential role of the protozoon in transmitting bacterial infection. Infect Immun 73(2):1180–1186. https://doi.org/10.1128/IAI.73.2.1180-1186.2005

Dessi D, Margarita V, Cocco AR, Marongiu A, Fiori PL, Rappelli P (2019) Trichomonas vaginalis and Mycoplasma hominis: new tales of two old friends. Parasitology:1–6. https://doi.org/10.1017/S0031182018002135

Dillon BJ, Holtsberg FW, Ensor CM, Bomalaski JS, Clark MA (2002) Biochemical characterization of the arginine degrading enzymes arginase and arginine deiminase and their effect on nitric oxide production. Med Sci Monit 8(7):BR248–BR253

Ding J, Wang K, Liu W, She Y, Sun Q, Shi J, Sun H, Wang D-C, Shao F (2016) Pore-forming activity and structural autoinhibition of the gasdermin family. Nature 535(7610):111–116. https://doi.org/10.1038/nature18590

Draper D, Jones W, Heine RP, Beutz M, French JI, McGregor JA (1995) Trichomonas vaginalis Weakens human Amniochorion in an in vitro model of premature membrane rupture. Infect Dis Obstet Gynecol 2(6):267–274. https://doi.org/10.1155/s1064744995000160

Edmondson MA, Joiner KS, Spencer JA, Riddell KP, Rodning SP, Gard JA, Givens MD (2017) Impact of a killed Tritrichomonas foetus vaccine on clearance of the organism and subsequent fertility of heifers following experimental inoculation. Theriogenology 90:245–251. https://doi.org/10.1016/j.theriogenology.2016.09.056

Fader CM, Sanchez DG, Mestre MB, Colombo MI (2009) TI-VAMP/VAMP7 and VAMP3/cellubrevin: two v-SNARE proteins involved in specific steps of the autophagy/multivesicular body pathways. Biochim Biophys Acta 1793(12):1901–1916. https://doi.org/10.1016/j.bbamcr.2009.09.011

Fang YK, Huang KY, Huang PJ, Lin R, Chao M, Tang P (2014) Gene-expression analysis of cold-stress response in the sexually transmitted protist Trichomonas vaginalis. J Microbiol Immunol Infect. https://doi.org/10.1016/j.jmii.2014.07.013

Fastring DR, Amedee A, Gatski M, Clark RA, Mena LA, Levison J, Schmidt N, Rice J, Gustat J, Kissinger P (2014) Co-occurrence of Trichomonas vaginalis and bacterial vaginosis and vaginal shedding of HIV-1 RNA. Sex Transm Dis 41(3):173–179. https://doi.org/10.1097/OLQ.0000000000000089

Fettweis JM, Serrano MG, Huang B, Brooks JP, Glascock AL, Sheth NU, Vaginal Microbiome C, Strauss JF 3rd, Jefferson KK, Buck GA (2014) An emerging mycoplasma associated with trichomoniasis, vaginal infection and disease. PLoS One 9(10):e110943. https://doi.org/10.1371/journal.pone.0110943

Fichorova RN (2009) Impact of T. vaginalis infection on innate immune responses and reproductive outcome. J Reprod Immunol 83(1-2):185–189. https://doi.org/10.1016/j.jri.2009.08.007

Fichorova RN, Rheinwald JG, Anderson DJ (1997) Generation of papillomavirus-immortalized cell lines from normal human ectocervical, endocervical, and vaginal epithelium that maintain expression of tissue-specific differentiation proteins. Biol Reprod 57(4):847–855. https://doi.org/10.1095/biolreprod57.4.847

Fichorova RN, Trifonova RT, Gilbert RO, Costello CE, Hayes GR, Lucas JJ, Singh BN (2006) Trichomonas vaginalis lipophosphoglycan triggers a selective upregulation of cytokines by human female reproductive tract epithelial cells. Infect Immun 74(10):5773–5779. https://doi.org/10.1128/iai.00631-06

Fichorova RN, Yamamoto HS, Delaney ML, Onderdonk AB, Doncel GF (2011) Novel vaginal microflora colonization model providing new insight into microbicide mechanism of action. *mBio 2*(6):e00168-00111. https://doi.org/10.1128/mBio.00168-11

Fichorova RN, Lee Y, Yamamoto HS, Takagi Y, Hayes GR, Goodman RP, Chepa-Lotrea X, Buck OR, Murray R, Kula T, Beach DH, Singh BN, Nibert ML (2012) Endobiont viruses sensed by the human host - beyond conventional antiparasitic therapy. PLoS One 7(11):e48418. https://doi.org/10.1371/journal.pone.0048418

Fichorova RN, Buck OR, Yamamoto HS, Fashemi T, Dawood HY, Fashemi B, Hayes GR, Beach DH, Takagi Y, Delaney ML, Nibert ML, Singh BN, Onderdonk AB (2013) The villain team-up or how Trichomonas vaginalis and bacterial vaginosis alter innate immunity in concert. Sex Transm Infect 89(6):460–466. https://doi.org/10.1136/sextrans-2013-051052

Fichorova RN, Yamamoto HS, Fashemi T, Foley E, Ryan S, Beatty N, Dawood H, Hayes GR, St-Pierre G, Sato S, Singh BN (2016) Trichomonas vaginalis lipophosphoglycan exploits binding to galectin-1 and -3 to modulate epithelial immunity. J Biol Chem 291(2):998–1013. https://doi.org/10.1074/jbc.M115.651497

Fichorova R, Fraga J, Rappelli P, Fiori PL (2017) Trichomonas vaginalis infection in symbiosis with Trichomonasvirus and Mycoplasma. Res Microbiol 168(9–10):882–891. https://doi.org/10.1016/j.resmic.2017.03.005

Figueroa-Angulo EE, Rendon-Gandarilla FJ, Puente-Rivera J, Calla-Choque JS, Cardenas-Guerra RE, Ortega-Lopez J, Quintas-Granados LI, Alvarez-Sanchez ME, Arroyo R (2012) The effects of environmental factors on the virulence of Trichomonas vaginalis. Microbes Infect 14 (15):1411–1427. https://doi.org/10.1016/j.micinf.2012.09.004

Fiori PL, Rappelli P, Rocchigiani AM, Cappuccinelli P (1993) Trichomonas vaginalis haemolysis: evidence of functional pores formation on red cell membranes. FEMS Microbiol Lett 109 (1):13–18. https://doi.org/10.1111/j.1574-6968.1993.tb06136.x

Fiori PL, Rappelli P, Addis MF, Sechi A, Cappuccinelli P (1996) Trichomonas vaginalis haemolysis: pH regulates a contact-independent mechanism based on pore-forming proteins. *Microb Pathog 20*(2):109–118. https://doi.org/10.1006/mpat.1996.0010

Fiori PL, Rappelli P, Addis MF, Mannu F, Cappuccinelli P (1997) Contact-dependent disruption of the host cell membrane skeleton induced by Trichomonas vaginalis. Infect Immun 65 (12):5142–5148

Fiori PL, Diaz N, Cocco AR, Rappelli P, Dessi D (2013) Association of Trichomonas vaginalis with its symbiont Mycoplasma hominis synergistically upregulates the in vitro proinflammatory response of human monocytes. Sex Transm Infect 89(6):449–454. https://doi.org/10.1136/sextrans-2012-051006

Ford SA, Kao D, Williams D, King KC (2016) Microbe-mediated host defence drives the evolution of reduced pathogen virulence. Nat Commun 7:13430. https://doi.org/10.1038/ncomms13430

Forna F, Gulmezoglu AM (2000) Interventions for treating trichomoniasis in women. Cochrane Database Syst Rev 3:CD000218. https://doi.org/10.1002/14651858.CD000218

Forna F, Gulmezoglu AM (2003) Interventions for treating trichomoniasis in women. Cochrane Database Syst Rev 2:CD000218. https://doi.org/10.1002/14651858.CD000218

Fraga J, Rojas L, Sariego I, Fernandez-Calienes A, Nunez FA (2012) Species typing of Cuban Trichomonas vaginalis virus by RT-PCR, and association of TVV-2 with high parasite adhesion levels and high pathogenicity in patients. Arch Virol 157(9):1789–1795. https://doi.org/10.1007/s00705-012-1353-4

Friedman M, Tam CC, Cheng LW, Land KM (2020) Anti-trichomonad activities of different compounds from foods, marine products, and medicinal plants: a review. BMC Complement Med Ther 20(1):271. https://doi.org/10.1186/s12906-020-03061-9

Furtado MB, Benchimol M (1998) Observation of membrane fusion on the interaction of Trichomonas vaginalis with human vaginal epithelial cells. Parasitol Res 84(3):213–220. https://doi.org/10.1007/s004360050385

Gajer P, Brotman RM, Bai G, Sakamoto J, Schutte UM, Zhong X, Koenig SS, Fu L, Ma ZS, Zhou X, Abdo Z, Forney LJ, Ravel J (2012) Temporal dynamics of the human vaginal microbiota. *Sci Transl Med 4*(132):132ra152. https://doi.org/10.1126/scitranslmed.3003605

Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P, Alnemri ES, Altucci L, Amelio I, Andrews DW, Annicchiarico-Petruzzelli M, Antonov AV, Arama E, Baehrecke EH, Barlev NA, Bazan NG, Bernassola F, Bertrand MJM, Bianchi K, Blagosklonny MV, Blomgren K, Borner C, Boya P, Brenner C, Campanella M, Candi E, Carmona-Gutierrez D, Cecconi F, Chan FK, Chandel NS, Cheng EH, Chipuk JE, Cidlowski JA, Ciechanover A, Cohen GM, Conrad M, Cubillos-Ruiz JR, Czabotar PE, D'Angiolella V, Dawson TM, Dawson VL, De Laurenzi V, De Maria R, Debatin KM, DeBerardinis RJ, Deshmukh M, Di Daniele N, Di Virgilio F, Dixit VM, Dixon SJ, Duckett CS, Dynlacht BD, El-Deiry WS, Elrod JW, Fimia GM, Fulda S, Garcia-Saez AJ, Garg AD, Garrido C, Gavathiotis E, Golstein P, Gottlieb E, Green DR, Greene LA, Gronemeyer H, Gross A, Hajnoczky G, Hardwick JM, Harris IS, Hengartner MO, Hetz C, Ichijo H, Jaattela M, Joseph B, Jost PJ, Juin PP, Kaiser WJ, Karin M, Kaufmann T, Kepp O, Kimchi A, Kitsis RN, Klionsky DJ, Knight RA, Kumar S, Lee SW, Lemasters JJ, Levine B, Linkermann A, Lipton SA, Lockshin RA, Lopez-Otin C, Lowe SW, Luedde T, Lugli E, MacFarlane M, Madeo F, Malewicz M, Malorni W, Manic G, Marine JC, Martin SJ, Martinou JC, Medema JP, Mehlen P, Meier P, Melino S, Miao EA, Molkentin JD, Moll UM, Munoz-Pinedo C, Nagata S, Nunez G, Oberst A, Oren M, Overholtzer M, Pagano M, Panaretakis T, Pasparakis M, Penninger JM, Pereira DM, Pervaiz S, Peter ME, Piacentini M, Pinton P, Prehn JHM, Puthalakath H, Rabinovich GA, Rehm M, Rizzuto R, Rodrigues CMP, Rubinsztein DC, Rudel T, Ryan KM, Sayan E, Scorrano L, Shao F, Shi Y, Silke J, Simon HU, Sistigu A, Stockwell BR, Strasser A, Szabadkai G, Tait SWG, Tang D, Tavernarakis N, Thorburn A, Tsujimoto Y, Turk B, Vanden Berghe T, Vandenabeele P, Vander Heiden MG, Villunger A, Virgin HW, Vousden KH, Vucic D, Wagner EF, Walczak H, Wallach D, Wang Y, Wells JA, Wood W, Yuan J, Zakeri Z, Zhivotovsky B, Zitvogel L, Melino G, Kroemer G (2018) Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death Differ 25(3):486–541. https://doi.org/10.1038/s41418-017-0012-4

Garcia AF, Benchimol M, Alderete JF (2005) Trichomonas vaginalis polyamine metabolism is linked to host cell adherence and cytotoxicity. Infect Immun 73(5):2602–2610. https://doi.org/10.1128/iai.73.5.2602-2610.2005

Garlanda C, Dinarello CA, Mantovani A (2013) The interleukin-1 family: back to the future. Immunity 39(6):1003–1018. https://doi.org/10.1016/j.immuni.2013.11.010

Ghosh I, Muwonge R, Mittal S, Banerjee D, Kundu P, Mandal R, Biswas J, Basu P (2017) Association between high risk human papillomavirus infection and co-infection with Candida spp. and Trichomonas vaginalis in women with cervical premalignant and malignant lesions. J Clin Virol 87:43–48. https://doi.org/10.1016/j.jcv.2016.12.007

Ghosh AP, Aycock C, Schwebke JR (2018) Susceptibility of clinical isolates of Trichomonas vaginalis to metronidazole and secnidazole-an in vitro study. Antimicrob Agents Chemother. https://doi.org/10.1128/AAC.02329-17

Gilbert RO, Elia G, Beach DH, Klaessig S, Singh BN (2000) Cytopathogenic effect of Trichomonas vaginalis on human vaginal epithelial cells cultured in vitro. Infect Immun 68(7):4200–4206. https://doi.org/10.1128/iai.68.7.4200-4206.2000

Gilbert NM, Lewis WG, Li G, Sojka DK, Lubin JB, Lewis AL (2019) Gardnerella vaginalis and prevotella bivia trigger distinct and overlapping phenotypes in a mouse model of bacterial vaginosis. J Infect Dis 220(7):1099–1108. https://doi.org/10.1093/infdis/jiy704

Gillin FD, Sher A (1981) Activation of the alternative complement pathway by Trichomonas vaginalis. Infect Immun 34(1):268–273

Gomez-Conde E, Mena-Lopez R, Hernandez-Jauregui P, Gonzalez-Camacho M, Arroyo R (2000) Trichomonas vaginalis: chromatin and mitotic spindle during mitosis. Exp Parasitol 96 (3):130–138. https://doi.org/10.1006/expr.2000.4551

Gomez-Lopez N, Laresgoiti-Servitje E, Olson DM, Estrada-Gutierrez G, Vadillo-Ortega F (2010) The role of chemokines in term and premature rupture of the fetal membranes: a review. Biol Reprod 82(5):809–814. https://doi.org/10.1095/biolreprod.109.080432

Goodman RP, Ghabrial SA, Fichorova RN, Nibert ML (2011) Trichomonasvirus: a new genus of protozoan viruses in the family Totiviridae. Arch Virol 156(1):171–179. https://doi.org/10.1007/s00705-010-0832-8

Goret J, Le Roy C, Touati A, Mesureur J, Renaudin H, Claverol S, Bebear C, Beven L, Pereyre S (2016) Surface lipoproteome of Mycoplasma hominis PG21 and differential expression after contact with human dendritic cells. Future Microbiol 11(2):179–194. https://doi.org/10.2217/fmb.15.130

Gould SB, Woehle C, Kusdian G, Landan G, Tachezy J, Zimorski V, Martin WF (2013) Deep sequencing of Trichomonas vaginalis during the early infection of vaginal epithelial cells and amoeboid transition. Int J Parasitol 43(9):707–719. https://doi.org/10.1016/j.ijpara.2013.04.002

Gray RH, Kigozi G, Serwadda D, Makumbi F, Nalugoda F, Watya S, Moulton L, Chen MZ, Sewankambo NK, Kiwanuka N, Sempijja V, Lutalo T, Kagayii J, Wabwire-Mangen F, Ridzon R, Bacon M, Wawer MJ (2009) The effects of male circumcision on female partners' genital tract symptoms and vaginal infections in a randomized trial in Rakai, Uganda. *Am J Obstet Gynecol 200*(1):42 e41–42 e47. https://doi.org/10.1016/j.ajog.2008.07.069

Gu NY, Kim JH, Han IH, Im SJ, Seo MY, Chung YH, Ryu JS (2016) Trichomonas vaginalis induces IL-1beta production in a human prostate epithelial cell line by activating the NLRP3 inflammasome via reactive oxygen species and potassium ion efflux. Prostate 76(10):885–896. https://doi.org/10.1002/pros.23178

Guenthner PC, Secor WE, Dezzutti CS (2005) Trichomonas vaginalis-induced epithelial monolayer disruption and human immunodeficiency virus type 1 (HIV-1) replication: implications for the sexual transmission of HIV-1. Infect Immun 73(7):4155–4160. https://doi.org/10.1128/IAI.73.7.4155-4160.2005

Hamilton H, Pontiff KL, Bolton M, Bradbury RS, Mathison BA, Bishop H, de Almeida M, Ogden BW, Barnett E, Rastanis D, Klar AL, Uzodi AS (2018) Trichomonas vaginalis brain abscess in a neonate. Clin Infect Dis 66(4):604–607. https://doi.org/10.1093/cid/cix908

Handrich MR, Garg SG, Sommerville EW, Hirt RP, Gould SB (2019) Characterization of the BspA and Pmp protein family of trichomonads. Parasit Vectors 12(1):406. https://doi.org/10.1186/s13071-019-3660-z

He WT, Wan H, Hu L, Chen P, Wang X, Huang Z, Yang ZH, Zhong CQ, Han J (2015) Gasdermin D is an executor of pyroptosis and required for interleukin-1beta secretion. Cell Res 25 (12):1285–1298. https://doi.org/10.1038/cr.2015.139

Heidary S, Bandehpour M, Valadkhani Z, Seyyed-Tabaee S, Haghighi A, Abadi A, Kazemi B (2013) Double-stranded RNA viral infection in Tehran Trichomonas vaginalis isolates. Iran J Parasitol 8(1):60–64

Henrich B, Kitzerow A, Feldmann RC, Schaal H, Hadding U (1996) Repetitive elements of the Mycoplasma hominis adhesin p50 can be differentiated by monoclonal antibodies. Infect Immun 64(10):4027–4034

Herbst-Kralovetz MM, Pyles RB, Ratner AJ, Sycuro LK, Mitchell C (2016) New systems for studying intercellular interactions in bacterial vaginosis. J Infect Dis 214(Suppl 1):S6–S13. https://doi.org/10.1093/infdis/jiw130

Hernandez H, Sariego I, Garber G, Delgado R, Lopez O, Sarracent J (2004) Monoclonal antibodies against a 62 kDa proteinase of Trichomonas vaginalis decrease parasite cytoadherence to epithelial cells and confer protection in mice. Parasite Immunol 26(3):119–125. https://doi.org/10.1111/j.0141-9838.2004.00690.x

Hernandez HM, Marcet R, Sarracent J (2014) Biological roles of cysteine proteinases in the pathogenesis of Trichomonas vaginalis. Parasite 21:54. https://doi.org/10.1051/parasite/2014054

Hernandez-Gutierrez R, Avila-Gonzalez L, Ortega-Lopez J, Cruz-Talonia F, Gomez-Gutierrez G, Arroyo R (2004) Trichomonas vaginalis: characterization of a 39-kDa cysteine proteinase found in patient vaginal secretions. Exp Parasitol 107(3-4):125–135. https://doi.org/10.1016/j.exppara.2004.05.004

Hernandez-Romano P, Hernandez R, Arroyo R, Alderete JF, Lopez-Villasenor I (2010) Identification and characterization of a surface-associated, subtilisin-like serine protease in Trichomonas vaginalis. Parasitology 137(11):1621–1635. https://doi.org/10.1017/s003118201000051x

Hinderfeld AS, Simoes-Barbosa A (2019) Vaginal dysbiotic bacteria act as pathobionts of the protozoal pathogen Trichomonas vaginalis. Microb Pathog 138:103820. https://doi.org/10.1016/j.micpath.2019.103820

Hinderfeld AS, Phukan N, Bar AK, Roberton AM, Simoes-Barbosa A (2019) Cooperative interactions between Trichomonas vaginalis and associated bacteria enhance paracellular permeability of the cervicovaginal epithelium by dysregulating tight junctions. Infect Immun 87(5):e00141-19. https://doi.org/10.1128/IAI.00141-19

Hirt RP, de Miguel N, Nakjang S, Dessi D, Liu YC, Diaz N, Rappelli P, Acosta-Serrano A, Fiori PL, Mottram JC (2011) Trichomonas vaginalis pathobiology new insights from the genome sequence. Adv Parasitol 77:87–140. https://doi.org/10.1016/B978-0-12-391429-3.00006-X

Hoang T, Toler E, DeLong K, Mafunda NA, Bloom SM, Zierden HC, Moench TR, Coleman JS, Hanes J, Kwon DS, Lai SK, Cone RA, Ensign LM (2020) The cervicovaginal mucus barrier to HIV-1 is diminished in bacterial vaginosis. PLoS Pathog 16(1):e1008236. https://doi.org/10.1371/journal.ppat.1008236

Hoffman CM, Fritz L, Radebe O, Dubbink JH, McIntyre JA, Kock MM, Peters RP (2018) Rectal Trichomonas vaginalis infection in South African men who have sex with men. Int J STD AIDS 29(14):1444–1447. https://doi.org/10.1177/0956462418788418

Honigberg BM, Volkmann D, Entzeroth R, Scholtyseck E (1984) A freeze-fracture electron microscope study of Trichomonas vaginalis Donne and Tritrichomonas foetus (Riedmuller). J Protozool 31(1):116–131. https://doi.org/10.1111/j.1550-7408.1984.tb04300.x

Hopfe M, Hoffmann R, Henrich B (2004) P80, the HinT interacting membrane protein, is a secreted antigen of Mycoplasma hominis. BMC Microbiol 4:46. https://doi.org/10.1186/1471-2180-4-46

Horvathova L, Safarikova L, Basler M, Hrdy I, Campo NB, Shin JW, Huang KY, Huang PJ, Lin R, Tang P, Tachezy J (2012) Transcriptomic identification of iron-regulated and iron-independent gene copies within the heavily duplicated Trichomonas vaginalis genome. Genome Biol Evol 4(10):1017–1029. https://doi.org/10.1093/gbe/evs078

Howe L, Wiggins R, Soothill PW, Millar MR, Horner PJ, Corfield AP (1999) Mucinase and sialidase activity of the vaginal microflora: implications for the pathogenesis of preterm labour. Int J STD AIDS 10(7):442–447. https://doi.org/10.1258/0956462991914438

Hrdy I, Cammack R, Stopka P, Kulda J, Tachezy J (2005) Alternative pathway of metronidazole activation in Trichomonas vaginalis hydrogenosomes. Antimicrob Agents Chemother 49(12):5033–5036. https://doi.org/10.1128/aac.49.12.5033-5036.2005

Huang KY, Chen YY, Fang YK, Cheng WH, Cheng CC, Chen YC, Wu TE, Ku FM, Chen SC, Lin R, Tang P (2014) Adaptive responses to glucose restriction enhance cell survival, antioxidant capability, and autophagy of the protozoan parasite Trichomonas vaginalis. Biochim Biophys Acta 1840(1):53–64. https://doi.org/10.1016/j.bbagen.2013.08.008

Huang KY, Ku FM, Cheng WH, Lee CC, Huang PJ, Chu LJ, Cheng CC, Fang YK, Wu HH, Tang P (2015) Novel insights into the molecular events linking to cell death induced by tetracycline in the amitochondriate protozoan Trichomonas vaginalis. Antimicrob Agents Chemother 59(11):6891–6903. https://doi.org/10.1128/AAC.01779-15

Huang KY, Ong SC, Wu CC, Hsu CW, Lin HC, Fang YK, Cheng WH, Huang PJ, Chiu CH, Tang P (2019) Metabolic reprogramming of hydrogenosomal amino acids in Trichomonas vaginalis

under glucose restriction. J Microbiol Immunol Infect 52(4):630–637. https://doi.org/10.1016/j.jmii.2017.10.005

Ioannidis A, Papaioannou P, Magiorkinis E, Magana M, Ioannidou V, Tzanetou K, Burriel AR, Tsironi M, Chatzipanagiotou S (2017) Detecting the diversity of mycoplasma and ureaplasma endosymbionts hosted by Trichomonas vaginalis isolates. Front Microbiol 8:1188. https://doi.org/10.3389/fmicb.2017.01188

Iriarte LS, Midlej V, Frontera LS, Moros Duarte D, Barbeito CG, de Souza W, Benchimol M, de Miguel N, Coceres VM (2018) TfVPS32 regulates cell division in the parasite Tritrichomonas foetus. J Eukaryot Microbiol 65(1):28–37. https://doi.org/10.1111/jeu.12424

Jang KS, Han IH, Lee SJ, Yoo J, Kim YS, Sim S, Ryu JS (2019) Experimental rat prostatitis caused by Trichomonas vaginalis infection. Prostate 79(4):379–389. https://doi.org/10.1002/pros.23744

Janssen BD, Chen YP, Molgora BM, Wang SE, Simoes-Barbosa A, Johnson PJ (2018) CRISPR/Cas9-mediated gene modification and gene knock out in the human-infective parasite Trichomonas vaginalis. Sci Rep 8(1):270. https://doi.org/10.1038/s41598-017-18442-3

Jarrett OD, Brady KE, Modur SP, Plants J, Landay AL, Ghassemi M, Golub ET, Spear GT, Novak RM (2015) T. vaginalis infection is associated with increased IL-8 and TNFr1 levels but with the absence of CD38 and HLADR activation in the Cervix of ESN. *PLoS One 10*(6):e0130146. https://doi.org/10.1371/journal.pone.0130146

Javurek AB, Spollen WG, Ali AM, Johnson SA, Lubahn DB, Bivens NJ, Bromert KH, Ellersieck MR, Givan SA, Rosenfeld CS (2016) Discovery of a novel seminal fluid microbiome and influence of estrogen receptor alpha genetic status. Sci Rep 6:23027. https://doi.org/10.1038/srep23027

Jemilohun PF (1998) Isolation and characterization of flagella from Trichomonas vaginalis. Parasitol Res 84(10):800–805. https://doi.org/10.1007/s004360050491

Juliano C, Rubino S, Zicconi D, Cappuccinelli P (1986) An immunofluorescent study of the microtubule organization in Trichomonas vaginalis using antitubulin antibodies. J Protozool 33(1):56–59. https://doi.org/10.1111/j.1550-7408.1986.tb05557.x

Kaur S, Khurana S, Bagga R, Wanchu A, Malla N (2008) Antitrichomonas IgG, IgM, IgA, and IgG subclass responses in human intravaginal trichomoniasis. Parasitol Res 103(2):305–312. https://doi.org/10.1007/s00436-008-0971-y

Kissinger P (2015) Trichomonas vaginalis: a review of epidemiologic, clinical and treatment issues. BMC Infect Dis 15:307. https://doi.org/10.1186/s12879-015-1055-0

Kitty Carter-Wicker, OU, Folashade Omole. (2016). Can trichomoniasis cause pharyngitis? A case report. SAGE open medical case reports

Kitzerow A, Hadding U, Henrich B (1999) Cyto-adherence studies of the adhesin P50 of Mycoplasma hominis. J Med Microbiol 48(5):485–493. https://doi.org/10.1099/00222615-48-5-485

Klebanoff MA, Carey JC, Hauth JC, Hillier SL, Nugent RP, Thom EA, Ernest JM, Heine RP, Wapner RJ, Trout W, Moawad A, Leveno KJ, Miodovnik M, Sibai BM, Van Dorsten JP, Dombrowski MP, O'Sullivan MJ, Varner M, Langer O, McNellis D, Roberts JM, National Institute of Child, H, & Human Development Network of Maternal-Fetal Medicine, U (2001) Failure of metronidazole to prevent preterm delivery among pregnant women with asymptomatic Trichomonas vaginalis infection. N Engl J Med 345(7):487–493. https://doi.org/10.1056/NEJMoa003329

Kolaczkowska E, Kubes P (2013) Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol 13(3):159–175. https://doi.org/10.1038/nri3399

Kovacs SB, Miao EA (2017) Gasdermins: effectors of pyroptosis. Trends Cell Biol 27(9):673–684. https://doi.org/10.1016/j.tcb.2017.05.005

Kowal J, Arras G, Colombo M, Jouve M, Morath JP, Primdal-Bengtson B, Dingli F, Loew D, Tkach M, Thery C (2016) Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. Proc Natl Acad Sci U S A 113(8):E968–E977. https://doi.org/10.1073/pnas.1521230113

Kuehn MJ, Kesty NC (2005) Bacterial outer membrane vesicles and the host-pathogen interaction. Genes Dev 19(22):2645–2655. https://doi.org/10.1101/gad.1299905

Kulp A, Kuehn MJ (2010) Biological functions and biogenesis of secreted bacterial outer membrane vesicles. Annu Rev Microbiol 64:163–184. https://doi.org/10.1146/annurev.micro.091208.073413

Kummer S, Hayes GR, Gilbert RO, Beach DH, Lucas JJ, Singh BN (2008) Induction of human host cell apoptosis by Trichomonas vaginalis cysteine proteases is modulated by parasite exposure to iron. Microb Pathog 44(3):197–203. https://doi.org/10.1016/j.micpath.2007.09.004

Lazenby GB, Soper DE, Nolte FS (2013) Correlation of leukorrhea and Trichomonas vaginalis infection. J Clin Microbiol 51(7):2323–2327. https://doi.org/10.1128/JCM.00416-13

Lee KE, Kim JH, Jung MK, Arii T, Ryu JS, Han SS (2009) Three-dimensional structure of the cytoskeleton in Trichomonas vaginalis revealed new features. J Electron Microsc 58 (5):305–313. https://doi.org/10.1093/jmicro/dfp019

Lehker MW, Sweeney D (1999) Trichomonad invasion of the mucous layer requires adhesins, mucinases, and motility. Sex Transm Infect 75(4):231–238. https://doi.org/10.1136/sti.75.4.231

Lehker MW, Chang TH, Dailey DC, Alderete JF (1990) Specific erythrocyte binding is an additional nutrient acquisition system for Trichomonas vaginalis. J Exp Med 171 (6):2165–2170. https://doi.org/10.1084/jem.171.6.2165

Leitsch D (2016) Recent advances in the Trichomonas vaginalis field. F1000Res 5:162. https://doi.org/10.12688/f1000research.7594.1

Leitsch D, Drinic M, Kolarich D, Duchene M (2012) Down-regulation of flavin reductase and alcohol dehydrogenase-1 (ADH1) in metronidazole-resistant isolates of Trichomonas vaginalis. Mol Biochem Parasitol 183(2):177–183. https://doi.org/10.1016/j.molbiopara.2012.03.003

Lev-Sagie A, Goldman-Wohl D, Cohen Y, Dori-Bachash M, Leshem A, Mor U, Strahilevitz J, Moses AE, Shapiro H, Yagel S, Elinav E (2019) Vaginal microbiome transplantation in women with intractable bacterial vaginosis. Nat Med 25(10):1500–1504. https://doi.org/10.1038/s41591-019-0600-6

Lewis WG, Robinson LS, Gilbert NM, Perry JC, Lewis AL (2013) Degradation, foraging, and depletion of mucus sialoglycans by the vagina-adapted Actinobacterium Gardnerella vaginalis. J Biol Chem 288(17):12067–12079. https://doi.org/10.1074/jbc.M113.453654

Li Y, Di Santo JP (2019) Modeling infectious diseases in mice with a "humanized" immune system. Microbiol Spectr 7(2). https://doi.org/10.1128/microbiolspec.BAI-0019-2019

Limberger R, Wickham SA (2011) Competition-colonization trade-offs in a ciliate model community. Oecologia 167(3):723–732. https://doi.org/10.1007/s00442-011-2013-1

Lin WC, Chang WT, Chang TY, Shin JW (2015) The pathogenesis of human cervical epithelium cells induced by interacting with Trichomonas vaginalis. PLoS One 10(4):e0124087. https://doi.org/10.1371/journal.pone.0124087

Liu X, Zhang Z, Ruan J, Pan Y, Magupalli VG, Wu H, Lieberman J (2016) Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. Nature 535 (7610):153–158. https://doi.org/10.1038/nature18629

Lizarraga A, O'Brown ZK, Boulias K, Roach L, Greer EL, Johnson PJ, Strobl-Mazzulla PH, de Miguel N (2020) Adenine DNA methylation, 3D genome organization, and gene expression in the parasite Trichomonas vaginalis. Proc Natl Acad Sci U S A 117(23):13033–13043. https://doi.org/10.1073/pnas.1917286117

Lustig G, Ryan CM, Secor WE, Johnson PJ (2013) Trichomonas vaginalis contact-dependent cytolysis of epithelial cells. Infect Immun 81(5):1411–1419. https://doi.org/10.1128/IAI.01244-12

Ma L, Meng Q, Cheng W, Sung Y, Tang P, Hu S, Yu J (2011) Involvement of the GP63 protease in infection of Trichomonas vaginalis. Parasitol Res 109(1):71–79. https://doi.org/10.1007/s00436-010-2222-2

Machado A, Cerca N (2015) Influence of Biofilm Formation by Gardnerella vaginalis and Other Anaerobes on Bacterial Vaginosis. J Infect Dis 212(12):1856–1861. https://doi.org/10.1093/infdis/jiv338

Makinde HM, Zariffard R, Mirmonsef P, Novak RM, Jarrett O, Landay AL, Spear GT (2013) IL-22 levels are associated with Trichomonas vaginalis infection in the lower genital tract. Am J Reprod Immunol 70(1):38–44. https://doi.org/10.1111/aji.12100

Man SM, Kanneganti TD (2016) Converging roles of caspases in inflammasome activation, cell death and innate immunity. Nat Rev Immunol 16(1):7–21. https://doi.org/10.1038/nri.2015.7

Margarita V, Rappelli P, Dessi D, Pintus G, Hirt RP, Fiori PL (2016) Symbiotic association with mycoplasma hominis can influence growth rate, ATP production, cytolysis and inflammatory response of Trichomonas vaginalis. Front Microbiol 7:953. https://doi.org/10.3389/fmicb.2016.00953

Marous M, Huang WY, Rabkin CS, Hayes RB, Alderete JF, Rosner B, Grubb RL 3rd, Winter AC, Sutcliffe S (2017) Trichomonas vaginalis infection and risk of prostate cancer: associations by disease aggressiveness and race/ethnicity in the PLCO Trial. Cancer Causes Control 28 (8):889–898. https://doi.org/10.1007/s10552-017-0919-6

Martin DH, Zozaya M, Lillis RA, Myers L, Nsuami MJ, Ferris MJ (2013) Unique vaginal microbiota that includes an unknown Mycoplasma-like organism is associated with Trichomonas vaginalis infection. J Infect Dis 207(12):1922–1931. https://doi.org/10.1093/infdis/jit100

Masha SC, Cools P, Descheemaeker P, Reynders M, Sanders EJ, Vaneechoutte M (2018) Urogenital pathogens, associated with Trichomonas vaginalis, among pregnant women in Kilifi, Kenya: a nested case-control study. BMC Infect Dis 18(1):549. https://doi.org/10.1186/s12879-018-3455-4

Mathieu M, Martin-Jaular L, Lavieu G, Thery C (2019) Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. Nat Cell Biol 21 (1):9–17. https://doi.org/10.1038/s41556-018-0250-9

Mathivanan S, Lim JW, Tauro BJ, Ji H, Moritz RL, Simpson RJ (2010) Proteomics analysis of A33 immunoaffinity-purified exosomes released from the human colon tumor cell line LIM1215 reveals a tissue-specific protein signature. Mol Cell Proteomics 9(2):197–208. https://doi.org/10.1074/mcp.M900152-MCP200

McClelland RS, Sangare L, Hassan WM, Lavreys L, Mandaliya K, Kiarie J, Ndinya-Achola J, Jaoko W, Baeten JM (2007) Infection with Trichomonas vaginalis increases the risk of HIV-1 acquisition. J Infect Dis 195(5):698–702. https://doi.org/10.1086/511278

McGregor JA, French JI, Jones W, Milligan K, McKinney PJ, Patterson E, Parker R (1994) Bacterial vaginosis is associated with prematurity and vaginal fluid mucinase and sialidase: results of a controlled trial of topical clindamycin cream. Am J Obstet Gynecol 170 (4):1048–1059; discussion 1059–1060. https://doi.org/10.1016/s0002-9378(94)70098-2

Meade JC, Carlton JM (2013) Genetic diversity in Trichomonas vaginalis. Sex Transm Infect 89 (6):444–448. https://doi.org/10.1136/sextrans-2013-051098

Mehta SD, Moses S, Agot K, Parker C, Ndinya-Achola JO, Maclean I, Bailey RC (2009) Adult male circumcision does not reduce the risk of incident Neisseria gonorrhoeae, Chlamydia trachomatis, or Trichomonas vaginalis infection: results from a randomized, controlled trial in Kenya. J Infect Dis 200(3):370–378. https://doi.org/10.1086/600074

Mendoza-Lopez MR, Becerril-Garcia C, Fattel-Facenda LV, Avila-Gonzalez L, Ruiz-Tachiquin ME, Ortega-Lopez J, Arroyo R (2000) CP30, a cysteine proteinase involved in Trichomonas vaginalis cytoadherence. Infect Immun 68(9):4907–4912. https://doi.org/10.1128/iai.68.9.4907-4912.2000

Mercer F, Johnson PJ (2018) Trichomonas vaginalis: pathogenesis, symbiont interactions, and host cell immune responses. Trends Parasitol 34(8):683–693. https://doi.org/10.1016/j.pt.2018.05.006

Mercer F, Diala FG, Chen YP, Molgora BM, Ng SH, Johnson PJ (2016) Leukocyte Lysis and Cytokine Induction by the Human Sexually Transmitted Parasite Trichomonas vaginalis. PLoS Negl Trop Dis 10(8):e0004913. https://doi.org/10.1371/journal.pntd.0004913

Mercer F, Ng SH, Brown TM, Boatman G, Johnson PJ (2018) Neutrophils kill the parasite Trichomonas vaginalis using trogocytosis. PLoS Biol 16(2):e2003885. https://doi.org/10.1371/journal.pbio.2003885

Meyer-Siegler KL, Iczkowski KA, Vera PL (2005) Further evidence for increased macrophage migration inhibitory factor expression in prostate cancer. BMC Cancer 5:73. https://doi.org/10.1186/1471-2407-5-73

Meyer-Siegler KL, Iczkowski KA, Leng L, Bucala R, Vera PL (2006) Inhibition of macrophage migration inhibitory factor or its receptor (CD74) attenuates growth and invasion of DU-145 prostate cancer cells. J Immunol 177(12):8730–8739. https://doi.org/10.4049/jimmunol.177.12.8730

Midlej V, Benchimol M (2010) Trichomonas vaginalis kills and eats--evidence for phagocytic activity as a cytopathic effect. Parasitology 137(1):65–76

Mielczarek E, Blaszkowska J (2016) Trichomonas vaginalis: pathogenicity and potential role in human reproductive failure. Infection 44(4):447–458. https://doi.org/10.1007/s15010-015-0860-0

Miranda-Ozuna JFT, Rivera-Rivas LA, Cardenas-Guerra RE, Hernandez-Garcia MS, Rodriguez-Cruz S, Gonzalez-Robles A, Chavez-Munguia B, Arroyo R (2019) Glucose-restriction increases Trichomonas vaginalis cellular damage towards HeLa cells and proteolytic activity of cysteine proteinases (CPs), such as TvCP2. Parasitology 146(9):1156–1166. https://doi.org/10.1017/s0031182019000209

Morada M, Manzur M, Lam B, Tan C, Tachezy J, Rappelli P, Dessi D, Fiori PL, Yarlett N (2010) Arginine metabolism in Trichomonas vaginalis infected with Mycoplasma hominis. Microbiology 156(Pt 12):3734–3743. https://doi.org/10.1099/mic.0.042192-0

Moreno I, Simon C (2019) Deciphering the effect of reproductive tract microbiota on human reproduction. Reprod Med Biol 18(1):40–50. https://doi.org/10.1002/rmb2.12249

Munoz C, Perez M, Orrego PR, Osorio L, Gutierrez B, Sagua H, Castillo JL, Martinez-Oyanedel J, Arroyo R, Meza-Cervantez P, da Silveira JF, Midlej V, Benchimol M, Cordero E, Morales P, Araya JE, Gonzalez J (2012) A protein phosphatase 1 gamma (PP1gamma) of the human protozoan parasite Trichomonas vaginalis is involved in proliferation and cell attachment to the host cell. Int J Parasitol 42(8):715–727. https://doi.org/10.1016/j.ijpara.2012.03.012

Muzny CA, Sunesara IR, Martin DH, Mena LA (2011) Sexually transmitted infections and risk behaviors among African American women who have sex with women: does sex with men make a difference? Sex Transm Dis 38(12):1118–1125. https://doi.org/10.1097/OLQ.0b013e31822e6179

Muzny CA, Rivers CA, Mena LA, Schwebke JR (2012) Genotypic characterization of Trichomonas vaginalis isolates among women who have sex with women in sexual partnerships. Sex Transm Dis 39(7):556–558. https://doi.org/10.1097/OLQ.0b013e31824f1c49

Muzny CA, Van Gerwen OT, Kissinger P (2020) Updates in trichomonas treatment including persistent infection and 5-nitroimidazole hypersensitivity. Curr Opin Infect Dis 33(1):73–77. https://doi.org/10.1097/QCO.0000000000000618

Najafi A, Chaechi Nosrati MR, Ghasemi E, Navi Z, Yousefi A, Majidiani H, Ghaneialvar H, Sayehmiri K, Galvan-Ramirez ML, Fakhar M (2019) Is there association between Trichomonas vaginalis infection and prostate cancer risk?: A systematic review and meta-analysis. Microb Pathog 137:103752. https://doi.org/10.1016/j.micpath.2019.103752

Nakjang S, Ndeh DA, Wipat A, Bolam DN, Hirt RP (2012) A novel extracellular metallopeptidase domain shared by animal host-associated mutualistic and pathogenic microbes. PLoS One 7(1):e30287. https://doi.org/10.1371/journal.pone.0030287

Nakubulwa S, Kaye DK, Bwanga F, Tumwesigye NM, Mirembe FM (2015) Genital infections and risk of premature rupture of membranes in Mulago Hospital, Uganda: a case control study. BMC Res Notes 8:573. https://doi.org/10.1186/s13104-015-1545-6

Nam YH, Min A, Kim SH, Lee YA, Kim KA, Song KJ, Shin MH (2012) Leukotriene B(4) receptors BLT1 and BLT2 are involved in interleukin-8 production in human neutrophils induced by Trichomonas vaginalis-derived secretory products. Inflamm Res 61(2):97–102. https://doi.org/10.1007/s00011-011-0425-3

Nievas YR, Coceres VM, Midlej V, de Souza W, Benchimol M, Pereira-Neves A, Vashisht AA, Wohlschlegel JA, Johnson PJ, de Miguel N (2018a) Membrane-shed vesicles from the parasite

Trichomonas vaginalis: characterization and their association with cell interaction. Cell Mol Life Sci 75(12):2211–2226. https://doi.org/10.1007/s00018-017-2726-3

Nievas YR, Vashisht AA, Corvi MM, Metz S, Johnson PJ, Wohlschlegel JA, de Miguel N (2018b) Protein palmitoylation plays an important role in Trichomonas vaginalis adherence. Mol Cell Proteomics. https://doi.org/10.1074/mcp.RA117.000018

Nievas YR, Lizarraga A, Salas N, Coceres VM, de Miguel N (2020) Extracellular vesicles released by anaerobic protozoan parasites: Current situation. Cell Microbiol 22(11):e13257. https://doi.org/10.1111/cmi.13257

Noel CJ, Diaz N, Sicheritz-Ponten T, Safarikova L, Tachezy J, Tang P, Fiori PL, Hirt RP (2010) Trichomonas vaginalis vast BspA-like gene family: evidence for functional diversity from structural organisation and transcriptomics. BMC Genomics 11:99

O'Donoghue AJ, Bibo-Verdugo B, Miyamoto Y, Wang SC, Yang JZ, Zuill DE, Matsuka S, Jiang Z, Almaliti J, Caffrey CR, Gerwick WH, Eckmann L (2019) 20S proteasome as a drug target in Trichomonas vaginalis. Antimicrob Agents Chemother 63(11):e00448-19. https://doi.org/10.1128/AAC.00448-19

O'Malley MA (2017) From endosymbiosis to holobionts: evaluating a conceptual legacy. J Theor Biol 434:34–41. https://doi.org/10.1016/j.jtbi.2017.03.008

Okumura CY, Baum LG, Johnson PJ (2008) Galectin-1 on cervical epithelial cells is a receptor for the sexually 9transmitted human parasite Trichomonas vaginalis. Cell Microbiol 10 (10):2078–2090. https://doi.org/10.1111/j.1462-5822.2008.01190.x

Olmos-Ortiz LM, Barajas-Mendiola MA, Barrios-Rodiles M, Castellano LE, Arias-Negrete S, Avila EE, Cuellar-Mata P (2017) Trichomonas vaginalis exosome-like vesicles modify the cytokine profile and reduce inflammation in parasite-infected mice. Parasite Immunol 39(6). https://doi.org/10.1111/pim.12426

Ostrowski M, Carmo NB, Krumeich S, Fanget I, Raposo G, Savina A, Moita CF, Schauer K, Hume AN, Freitas RP, Goud B, Benaroch P, Hacohen N, Fukuda M, Desnos C, Seabra MC, Darchen F, Amigorena S, Moita LF, Thery C (2010) Rab27a and Rab27b control different steps of the exosome secretion pathway. Nat Cell Biol 12(1):19–30; sup pp 11–13. https://doi.org/10.1038/ncb2000

Pachano T, Nievas YR, Lizarraga A, Johnson PJ, Strobl-Mazzulla PH, de Miguel N (2017) Epigenetics regulates transcription and pathogenesis in the parasite Trichomonas vaginalis. Cell Microbiol. https://doi.org/10.1111/cmi.12716

Padilla-Vaca F, Anaya-Velazquez F (1997) Biochemical properties of a neuraminidase of Trichomonas vaginalis. J Parasitol 83(6):1001–1006

Paintlia MK, Kaur S, Gupta I, Ganguly NK, Mahajan RC, Malla N (2002) Specific IgA response, T-cell subtype and cytokine profile in experimental intravaginal trichomoniasis. Parasitol Res 88 (4):338–343

Palomares RA, Hurley DJ, Crum LT, Rollin E, Collop T, Williard A, Felton J, Parrish J, Corbeil LB (2017) Serum, uterine, and vaginal mucosal IgG antibody responses against Tritrichomonas foetus after administration of a commercial killed whole T foetus vaccine in beef cows. Theriogenology 87:235–241. https://doi.org/10.1016/j.theriogenology.2016.08.031

Pararas MV, Skevaki CL, Kafetzis DA (2006) Preterm birth due to maternal infection: causative pathogens and modes of prevention. Eur J Clin Microbiol Infect Dis 25(9):562–569. https://doi.org/10.1007/s10096-006-0190-3

Pekmezovic M, Mogavero S, Naglik JR, Hube B (2019) Host-pathogen interactions during female genital tract infections. Trends Microbiol 27(12):982–996. https://doi.org/10.1016/j.tim.2019.07.006

Pereyre S, Sirand-Pugnet P, Beven L, Charron A, Renaudin H, Barre A, Avenaud P, Jacob D, Couloux A, Barbe V, de Daruvar A, Blanchard A, Bebear C (2009) Life on arginine for Mycoplasma hominis: clues from its minimal genome and comparison with other human urogenital mycoplasmas. PLoS Genet 5(10):e1000677. https://doi.org/10.1371/journal.pgen.1000677

Petrin D, Delgaty K, Bhatt R, Garber G (1998) Clinical and microbiological aspects of Trichomonas vaginalis. Clin Microbiol Rev 11(2):300–317

Phukan N, Parsamand T, Brooks AE, Nguyen TN, Simoes-Barbosa A (2013) The adherence of Trichomonas vaginalis to host ectocervical cells is influenced by lactobacilli. Sex Transm Infect 89(6):455–459. https://doi.org/10.1136/sextrans-2013-051039

Phukan N, Brooks AES, Simoes-Barbosa A (2018) A cell surface aggregation-promoting factor from Lactobacillus gasseri contributes towards inhibition of Trichomonas vaginalis adhesion to human vaginal ectocervical cells. Infect Immun. https://doi.org/10.1128/IAI.00907-17

Pindak FF, Mora de Pindak M, Gardner WA Jr (1993) Contact-independent cytotoxicity of Trichomonas vaginalis. Genitourin Med 69(1):35–40. https://doi.org/10.1136/sti.69.1.35

Pinheiro J, Biboy J, Vollmer W, Hirt RP, Keown JR, Artuyants A, Black MM, Goldstone DC, Simoes-Barbosa A (2018) The protozoan Trichomonas vaginalis targets bacteria with laterally acquired NlpC/P60 peptidoglycan hydrolases. *mBio* 9(6):e01784-18. https://doi.org/10.1128/mBio.01784-18

Pintye J, Drake AL, Unger JA, Matemo D, Kinuthia J, McClelland RS, John-Stewart G (2017) Male partner circumcision associated with lower Trichomonas vaginalis incidence among pregnant and postpartum Kenyan women: a prospective cohort study. Sex Transm Infect 93(2):137–143. https://doi.org/10.1136/sextrans-2016-052629

Polat ZA, Savage PB, Genberg C (2011) In vitro amoebicidal activity of a ceragenin, cationic steroid antibiotic-13, against Acanthamoeba castellanii and its cytotoxic potential. J Ocul Pharmacol Ther 27(1):1–5. https://doi.org/10.1089/jop.2010.0041

Provenzano D, Alderete JF (1995) Analysis of human immunoglobulin-degrading cysteine proteinases of Trichomonas vaginalis. Infect Immun 63(9):3388–3395

Puente-Rivera J, Villalpando JL, Villalobos-Osnaya A, Vazquez-Carrillo LI, Leon-Avila G, Ponce-Regalado MD, Lopez-Camarillo C, Elizalde-Contreras JM, Ruiz-May E, Arroyo R, Alvarez-Sanchez ME (2017) The 50kDa metalloproteinase TvMP50 is a zinc-mediated Trichomonas vaginalis virulence factor. Mol Biochem Parasitol 217:32–41. https://doi.org/10.1016/j.molbiopara.2017.09.001

Quan JH, Kang BH, Cha GH, Zhou W, Koh YB, Yang JB, Yoo HJ, Lee MA, Ryu JS, Noh HT, Kwon J, Lee YH (2014) Trichonomas vaginalis metalloproteinase induces apoptosis of SiHa cells through disrupting the Mcl-1/Bim and Bcl-xL/Bim complexes. PLoS One 9(10):e110659. https://doi.org/10.1371/journal.pone.0110659

Quan JH, Kang BH, Yang JB, Rhee YE, Noh HT, Choi IW, Cha GH, Yuk JM, Lee YH (2017) Trichomonas vaginalis induces SiHa cell apoptosis by NF-kappaB inactivation via reactive oxygen species. Biomed Res Int 2017:3904870. https://doi.org/10.1155/2017/3904870

Quintas-Granados LI, Villalpando JL, Vazquez-Carrillo LI, Arroyo R, Mendoza-Hernandez G, Alvarez-Sanchez ME (2013) TvMP50 is an immunogenic metalloproteinase during male trichomoniasis. Mol Cell Proteomics 12(7):1953–1964. https://doi.org/10.1074/mcp.M112.022012

Rai AK, Johnson PJ (2019) Trichomonas vaginalis extracellular vesicles are internalized by host cells using proteoglycans and caveolin-dependent endocytosis. Proc Natl Acad Sci U S A 116(43):21354–21360. https://doi.org/10.1073/pnas.1912356116

Ralston KS (2015a) Chew on this: amoebic trogocytosis and host cell killing by Entamoeba histolytica. Trends Parasitol 31(9):442–452. https://doi.org/10.1016/j.pt.2015.05.003

Ralston KS (2015b) Taking a bite: amoebic trogocytosis in Entamoeba histolytica and beyond. Curr Opin Microbiol 28:26–35. https://doi.org/10.1016/j.mib.2015.07.009

Ramon-Luing LA, Rendon-Gandarilla FJ, Cardenas-Guerra RE, Rodriguez-Cabrera NA, Ortega-Lopez J, Avila-Gonzalez L, Angel-Ortiz C, Herrera-Sanchez CN, Mendoza-Garcia M, Arroyo R (2010) Immunoproteomics of the active degradome to identify biomarkers for Trichomonas vaginalis. Proteomics 10(3):435–444. https://doi.org/10.1002/pmic.200900479

Rappelli P, Addis MF, Carta F, Fiori PL (1998) Mycoplasma hominis parasitism of Trichomonas vaginalis. Lancet 352(9136):1286. https://doi.org/10.1016/S0140-6736(98)00041-5

Rappelli P, Carta F, Delogu G, Addis MF, Dessi D, Cappuccinelli P, Fiori PL (2001) Mycoplasma hominis and Trichomonas vaginalis symbiosis: multiplicity of infection and transmissibility of M. hominis to human cells. Arch Microbiol 175(1):70–74

Rasmussen SE, Nielsen MH, Lind I, Rhodes JM (1986) Morphological studies of the cytotoxicity of Trichomonas vaginalis to normal human vaginal epithelial cells in vitro. Genitourin Med 62 (4):240–246. https://doi.org/10.1136/sti.62.4.240

Rathod SD, Krupp K, Klausner JD, Arun A, Reingold AL, Madhivanan P (2011) Bacterial vaginosis and risk for Trichomonas vaginalis infection: a longitudinal analysis. Sex Transm Dis 38(9):882–886. https://doi.org/10.1097/OLQ.0b013e31821f91a1

Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, Karlebach S, Gorle R, Russell J, Tacket CO, Brotman RM, Davis CC, Ault K, Peralta L, Forney LJ (2011) Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A 108(Suppl 1):4680–4687. https://doi.org/10.1073/pnas.1002611107

Razin S, Yogev D, Naot Y (1998) Molecular biology and pathogenicity of mycoplasmas. Microbiol Mol Biol Rev 62(4):1094–1156

Redondo-Lopez V, Cook RL, Sobel JD (1990) Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora. Rev Infect Dis 12(5):856–872. https://doi.org/10.1093/clinids/12.5.856

Rein MF, Sullivan JA, Mandell GL (1980) Trichomonacidal activity of human polymorphonuclear neutrophils: killing by disruption and fragmentation. J Infect Dis 142(4):575–585

Rendon-Gandarilla FJ, Ramon-Luing Lde L, Ortega-Lopez J, Rosa de Andrade I, Benchimol M, Arroyo R (2013) The TvLEGU-1, a legumain-like cysteine proteinase, plays a key role in Trichomonas vaginalis cytoadherence. Biomed Res Int 2013:561979. https://doi.org/10.1155/2013/561979

Rendon-Maldonado JG, Espinosa-Cantellano M, Gonzalez-Robles A, Martinez-Palomo A (1998) Trichomonas vaginalis: in vitro phagocytosis of lactobacilli, vaginal epithelial cells, leukocytes, and erythrocytes. Exp Parasitol 89(2):241–250. https://doi.org/10.1006/expr.1998.4297

Ribeiro KC, Monteiro-Leal LH, Benchimol M (2000) Contributions of the axostyle and flagella to closed mitosis in the protists Tritrichomonas foetus and Trichomonas vaginalis. J Eukaryot Microbiol 47(5):481–492. https://doi.org/10.1111/j.1550-7408.2000.tb00077.x

Ridley C, Thornton DJ (2018) Mucins: the frontline defence of the lung. Biochem Soc Trans 46 (5):1099–1106. https://doi.org/10.1042/BST20170402

Riestra AM, Gandhi S, Sweredoski MJ, Moradian A, Hess S, Urban S, Johnson PJ (2015) A Trichomonas vaginalis rhomboid protease and its substrate modulate parasite attachment and cytolysis of host cells. PLoS Pathog 11(12):e1005294. https://doi.org/10.1371/journal.ppat.1005294

Riestra AM, Valderrama JA, Patras KA, Booth SD, Quek XY, Tsai CM, Nizet V (2019) Trichomonas vaginalis induces NLRP3 inflammasome activation and pyroptotic cell death in human macrophages. J Innate Immun 11(1):86–98. https://doi.org/10.1159/000493585

Rivera-Rivas LA, Lorenzo-Benito S, Sanchez-Rodriguez DB, Miranda-Ozuna JF, Euceda-Padilla EA, Ortega-Lopez J, Chavez-Munguia B, Lagunes-Guillen A, Velazquez-Valassi B, Jasso-Villazul L, Arroyo R (2020) The effect of iron on Trichomonas vaginalis TvCP2, a cysteine proteinase found in vaginal secretions of trichomoniasis patients. Parasitology:1–52. https://doi.org/10.1017/s0031182020000438

Rogers C, Alnemri ES (2019) Gasdermins in apoptosis: new players in an old game. Yale J Biol Med 92(4):603–617

Rogers C, Erkes DA, Nardone A, Aplin AE, Fernandes-Alnemri T, Alnemri ES (2019) Gasdermin pores permeabilize mitochondria to augment caspase-3 activation during apoptosis and inflammasome activation. Nat Commun 10(1):1689. https://doi.org/10.1038/s41467-019-09397-2

Rosset I, Tasca T, Tessele PM, De Carli GA (2002) Scanning electron microscopy in the investigation of the in vitro hemolytic activity of Trichomonas vaginalis. Parasitol Res 88(4):356–359. https://doi.org/10.1007/s00436-001-0555-6

Ryan CM, de Miguel N, Johnson PJ (2011a) Trichomonas vaginalis: current understanding of host-parasite interactions. Essays Biochem 51:161–175. https://doi.org/10.1042/bse0510161

Ryan CM, Mehlert A, Richardson JM, Ferguson MA, Johnson PJ (2011b) Chemical structure of Trichomonas vaginalis surface lipoglycan: a role for short galactose (beta1-4/3) N-acetylglucosamine repeats in host cell interaction. J Biol Chem 286(47):40494–40508. https://doi.org/10.1074/jbc.M111.280578

Ryu JS, Kang JH, Jung SY, Shin MH, Kim JM, Park H, Min DY (2004) Production of interleukin-8 by human neutrophils stimulated with Trichomonas vaginalis. Infect Immun 72(3):1326–1332

Schmidt O, Teis D (2012) The ESCRT machinery. Curr Biol 22(4):R116–R120. https://doi.org/10.1016/j.cub.2012.01.028

Schneider RE, Brown MT, Shiflett AM, Dyall SD, Hayes RD, Xie Y, Loo JA, Johnson PJ (2011) The Trichomonas vaginalis hydrogenosome proteome is highly reduced relative to mitochondria, yet complex compared with mitosomes. Int J Parasitol 41(13–14):1421–1434. https://doi.org/10.1016/j.ijpara.2011.10.001

Secor WE, Meites E, Starr MC, Workowski KA (2014) Neglected parasitic infections in the United States: trichomoniasis. Am J Trop Med Hyg 90(5):800–804. https://doi.org/10.4269/ajtmh.13-0723

Seo MY, Im SJ, Gu NY, Kim JH, Chung YH, Ahn MH, Ryu JS (2014) Inflammatory response of prostate epithelial cells to stimulation by Trichomonas vaginalis. Prostate 74(4):441–449. https://doi.org/10.1002/pros.22766

Shaio MF, Lin PR, Liu JY, Tang KD (1994) Monocyte-derived interleukin-8 involved in the recruitment of neutrophils induced by Trichomonas vaginalis infection. J Infect Dis 170 (6):1638–1640

Shaio MF, Lin PR, Liu JY, Yang KD (1995) Generation of interleukin-8 from human monocytes in response to Trichomonas vaginalis stimulation. Infect Immun 63(10):3864–3870

Shi J, Zhao Y, Wang K, Shi X, Wang Y, Huang H, Zhuang Y, Cai T, Wang F, Shao F (2015) Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature 526 (7575):660–665. https://doi.org/10.1038/nature15514

Shiflett AM, Johnson PJ (2010) Mitochondrion-related organelles in eukaryotic protists. Annu Rev Microbiol 64:409–429. https://doi.org/10.1146/annurev.micro.62.081307.162826

Shui IM, Kolb S, Hanson C, Sutcliffe S, Rider JR, Stanford JL (2016) Trichomonas vaginalis infection and risk of advanced prostate cancer. Prostate 76(7):620–623. https://doi.org/10.1002/pros.23153

Sierra LJ, Brown AG, Barila GO, Anton L, Barnum CE, Shetye SS, Soslowsky LJ, Elovitz MA (2018) Colonization of the cervicovaginal space with Gardnerella vaginalis leads to local inflammation and cervical remodeling in pregnant mice. PLoS One 13(1):e0191524. https://doi.org/10.1371/journal.pone.0191524

Silver BJ, Guy RJ, Kaldor JM, Jamil MS, Rumbold AR (2014) Trichomonas vaginalis as a cause of perinatal morbidity: a systematic review and meta-analysis. Sex Transm Dis 41(6):369–376. https://doi.org/10.1097/OLQ.0000000000000134

Singer SM, Fink MY, Angelova VV (2019) Recent insights into innate and adaptive immune responses to Giardia. Adv Parasitol 106:171–208. https://doi.org/10.1016/bs.apar.2019.07.004

Singh BN, Beach DH, Lindmark DG, Costello CE (1994) Identification of the lipid moiety and further characterization of the novel lipophosphoglycan-like glycoconjugates of Trichomonas vaginalis and Trichomonas foetus. Arch Biochem Biophys 309(2):273–280

Slaats J, Ten Oever J, van de Veerdonk FL, Netea MG (2016) IL-1beta/IL-6/CRP and IL-18/ferritin: distinct inflammatory programs in infections. PLoS Pathog 12(12):e1005973. https://doi.org/10.1371/journal.ppat.1005973

Smith JD, Garber GE (2015) Trichomonas vaginalis infection induces vaginal CD4+ T-cell infiltration in a mouse model: a vaccine strategy to reduce vaginal infection and HIV transmission. J Infect Dis 212(2):285–293. https://doi.org/10.1093/infdis/jiv036

Smith BF, Stewart BT (1966) Fine structure of Trichomonas vaginalis. Exp Parasitol 19(1):52–63. https://doi.org/10.1016/0014-4894(66)90052-x

Smith GR, Steidinger BS, Bruns TD, Peay KG (2018) Competition-colonization tradeoffs structure fungal diversity. ISME J 12(7):1758–1767. https://doi.org/10.1038/s41396-018-0086-0

Sobngwi-Tambekou J, Taljaard D, Nieuwoudt M, Lissouba P, Puren A, Auvert B (2009) Male circumcision and Neisseria gonorrhoeae, Chlamydia trachomatis and Trichomonas vaginalis: observations after a randomised controlled trial for HIV prevention. Sex Transm Infect 85 (2):116–120. https://doi.org/10.1136/sti.2008.032334

Sommer U, Costello CE, Hayes GR, Beach DH, Gilbert RO, Lucas JJ, Singh BN (2005) Identification of Trichomonas vaginalis cysteine proteases that induce apoptosis in human vaginal epithelial cells. J Biol Chem 280(25):23853–23860. https://doi.org/10.1074/jbc.M501752200

Song HO, Shin MH, Ahn MH, Min DY, Kim YS, Ryu JS (2008) Trichomonas vaginalis: reactive oxygen species mediates caspase-3 dependent apoptosis of human neutrophils. Exp Parasitol 118(1):59–65. https://doi.org/10.1016/j.exppara.2007.06.010

Song MJ, Lee JJ, Nam YH, Kim TG, Chung YW, Kim M, Choi YE, Shin MH, Kim HP (2015) Modulation of dendritic cell function by Trichomonas vaginalis-derived secretory products. BMB Rep 48(2):103–108

Song MJ, Kim M, Choi Y, Yi MH, Kim J, Park SJ, Yong TS, Kim HP (2017) Epigenome mapping highlights chromatin-mediated gene regulation in the protozoan parasite Trichomonas vaginalis. Sci Rep 7:45365. https://doi.org/10.1038/srep45365

Stafkova J, Rada P, Meloni D, Zarsky V, Smutna T, Zimmann N, Harant K, Pompach P, Hrdy I, Tachezy J (2018) Dynamic secretome of Trichomonas vaginalis: case study of beta-amylases. Mol Cell Proteomics 17(2):304–320. https://doi.org/10.1074/mcp.RA117.000434

Štáfková J, Rada P, Meloni D, Žárský V, Smutná T, Zimmann N, Harant K, Pompach P, Hrdý I, Tachezy J (2018) Dynamic secretome of Trichomonas vaginalis: case study of β-amylases. Mol Cell Proteom 17(2):304–320. https://doi.org/10.1074/mcp.RA117.000434

Stark JR, Judson G, Alderete JF, Mundodi V, Kucknoor AS, Giovannucci EL, Platz EA, Sutcliffe S, Fall K, Kurth T, Ma J, Stampfer MJ, Mucci LA (2009) Prospective study of Trichomonas vaginalis infection and prostate cancer incidence and mortality: Physicians' Health Study. J Natl Cancer Inst 101(20):1406–1411. https://doi.org/10.1093/jnci/djp306

Sun T, Holowka T, Song Y, Zierow S, Leng L, Chen Y, Xiong H, Griffith J, Nouraie M, Thuma PE, Lolis E, Janse CJ, Gordeuk VR, Augustijn K, Bucala R (2012) A plasmodium-encoded cytokine suppresses T-cell immunity during malaria. Proc Natl Acad Sci U S A 109(31):E2117–E2126. https://doi.org/10.1073/pnas.1206573109

Sutcliffe S, Giovannucci E, Alderete JF, Chang TH, Gaydos CA, Zenilman JM, De Marzo AM, Willett WC, Platz EA (2006) Plasma antibodies against Trichomonas vaginalis and subsequent risk of prostate cancer. Cancer Epidemiol Biomark Prev 15(5):939–945. https://doi.org/10.1158/1055-9965.EPI-05-0781

Sutcliffe S, Neace C, Magnuson NS, Reeves R, Alderete JF (2012) Trichomonosis, a common curable STI, and prostate carcinogenesis--a proposed molecular mechanism. PLoS Pathog 8(8): e1002801. https://doi.org/10.1371/journal.ppat.1002801

Swidsinski A, Mendling W, Loening-Baucke V, Ladhoff A, Swidsinski S, Hale LP, Lochs H (2005) Adherent biofilms in bacterial vaginosis. Obstet Gynecol 106(5 Pt 1):1013–1023. https://doi.org/10.1097/01.AOG.0000183594.45524.d2

Tao L, Han L, Li X, Gao Q, Pan L, Wu L, Luo Y, Wang W, Zheng Z, Guo X (2014) Prevalence and risk factors for cervical neoplasia: a cervical cancer screening program in Beijing. BMC Public Health 14:1185. https://doi.org/10.1186/1471-2458-14-1185

Thery C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, Antoniou A, Arab T, Archer F, Atkin-Smith GK, Ayre DC, Bach JM, Bachurski D, Baharvand H, Balaj L, Baldacchino S, Bauer NN, Baxter AA, Bebawy M, Beckham C, Bedina Zavec A, Benmoussa A, Berardi AC, Bergese P, Bielska E, Blenkiron C, Bobis-Wozowicz S, Boilard E, Boireau W, Bongiovanni A, Borras FE, Bosch S, Boulanger CM, Breakefield X, Breglio AM, Brennan MA, Brigstock DR, Brisson A, Broekman ML, Bromberg JF, Bryl-Gorecka P, Buch S, Buck AH, Burger D, Busatto S, Buschmann D, Bussolati B, Buzas EI, Byrd JB, Camussi G, Carter DR, Caruso S, Chamley LW, Chang YT, Chen C, Chen S, Cheng L, Chin

AR, Clayton A, Clerici SP, Cocks A, Cocucci E, Coffey RJ, Cordeiro-da-Silva A, Couch Y, Coumans FA, Coyle B, Crescitelli R, Criado MF, D'Souza-Schorey C, Das S, Datta Chaudhuri A, de Candia P, De Santana EF, De Wever O, Del Portillo HA, Demaret T, Deville S, Devitt A, Dhondt B, Di Vizio D, Dieterich LC, Dolo V, Dominguez Rubio AP, Dominici M, Dourado MR, Driedonks TA, Duarte FV, Duncan HM, Eichenberger RM, Ekstrom K, El Andaloussi S, Elie-Caille C, Erdbrugger U, Falcon-Perez JM, Fatima F, Fish JE, Flores-Bellver M, Forsonits A, Frelet-Barrand A, Fricke F, Fuhrmann G, Gabrielsson S, Gamez-Valero A, Gardiner C, Gartner K, Gaudin R, Gho YS, Giebel B, Gilbert C, Gimona M, Giusti I, Goberdhan DC, Gorgens A, Gorski SM, Greening DW, Gross JC, Gualerzi A, Gupta GN, Gustafson D, Handberg A, Haraszti RA, Harrison P, Hegyesi H, Hendrix A, Hill AF, Hochberg FH, Hoffmann KF, Holder B, Holthofer H, Hosseinkhani B, Hu G, Huang Y, Huber V, Hunt S, Ibrahim AG, Ikezu T, Inal JM, Isin M, Ivanova A, Jackson HK, Jacobsen S, Jay SM, Jayachandran M, Jenster G, Jiang L, Johnson SM, Jones JC, Jong A, Jovanovic-Talisman T, Jung S, Kalluri R, Kano SI, Kaur S, Kawamura Y, Keller ET, Khamari D, Khomyakova E, Khvorova A, Kierulf P, Kim KP, Kislinger T, Klingeborn M, Klinke DJ II, Kornek M, Kosanovic MM, Kovacs AF, Kramer-Albers EM, Krasemann S, Krause M, Kurochkin IV, Kusuma GD, Kuypers S, Laitinen S, Langevin SM, Languino LR, Lannigan J, Lasser C, Laurent LC, Lavieu G, Lazaro-Ibanez E, Le Lay S, Lee MS, Lee YXF, Lemos DS, Lenassi M, Leszczynska A, Li IT, Liao K, Libregts SF, Ligeti E, Lim R, Lim SK, Line A, Linnemannstons K, Llorente A, Lombard CA, Lorenowicz MJ, Lorincz AM, Lotvall J, Lovett J, Lowry MC, Loyer X, Lu Q, Lukomska B, Lunavat TR, Maas SL, Malhi H, Marcilla A, Mariani J, Mariscal J, Martens-Uzunova ES, Martin-Jaular L, Martinez MC, Martins VR, Mathieu M, Mathivanan S, Maugeri M, McGinnis LK, McVey MJ, Meckes DG Jr, Meehan KL, Mertens I, Minciacchi VR, Moller A, Moller Jorgensen M, Morales-Kastresana A, Morhayim J, Mullier F, Muraca M, Musante L, Mussack V, Muth DC, Myburgh KH, Najrana T, Nawaz M, Nazarenko I, Nejsum P, Neri C, Neri T, Nieuwland R, Nimrichter L, Nolan JP, Nolte-'t Hoen EN, Noren Hooten N, O'Driscoll L, O'Grady T, O'Loghlen A, Ochiya T, Olivier M, Ortiz A, Ortiz LA, Osteikoetxea X, Ostergaard O, Ostrowski M, Park J, Pegtel DM, Peinado H, Perut F, Pfaffl MW, Phinney DG, Pieters BC, Pink RC, Pisetsky DS, Pogge von Strandmann E, Polakovicova I, Poon IK, Powell BH, Prada I, Pulliam L, Quesenberry P, Radeghieri A, Raffai RL, Raimondo S, Rak J, Ramirez MI, Raposo G, Rayyan MS, Regev-Rudzki N, Ricklefs FL, Robbins PD, Roberts DD, Rodrigues SC, Rohde E, Rome S, Rouschop KM, Rughetti A, Russell AE, Saa P, Sahoo S, Salas-Huenuleo E, Sanchez C, Saugstad JA, Saul MJ, Schiffelers RM, Schneider R, Schoyen TH, Scott A, Shahaj E, Sharma S, Shatnyeva O, Shekari F, Shelke GV, Shetty AK, Shiba K, Siljander PR, Silva AM, Skowronek A, Snyder OL II, Soares RP, Sodar BW, Soekmadji C, Sotillo J, Stahl PD, Stoorvogel W, Stott SL, Strasser EF, Swift S, Tahara H, Tewari M, Timms K, Tiwari S, Tixeira R, Tkach M, Toh WS, Tomasini R, Torrecilhas AC, Tosar JP, Toxavidis V, Urbanelli L, Vader P, van Balkom BW, van der Grein SG, Van Deun J, van Herwijnen MJ, Van Keuren-Jensen K, van Niel G, van Royen ME, van Wijnen AJ, Vasconcelos MH, Vechetti IJ Jr, Veit TD, Vella LJ, Velot E, Verweij FJ, Vestad B, Vinas JL, Visnovitz T, Vukman KV, Wahlgren J, Watson DC, Wauben MH, Weaver A, Webber JP, Weber V, Wehman AM, Weiss DJ, Welsh JA, Wendt S, Wheelock AM, Wiener Z, Witte L, Wolfram J, Xagorari A, Xander P, Xu J, Yan X, Yanez-Mo M, Yin H, Yuana Y, Zappulli V, Zarubova J, Zekas V, Zhang JY, Zhao Z, Zheng L, Zheutlin AR, Zickler AM, Zimmermann P, Zivkovic AM, Zocco D, Zuba-Surma EK (2018) Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles 7(1):1535750. doi:https://doi.org/10.1080/20013078.2018.1535750

Thi Trung Thu T, Margarita V, Cocco AR, Marongiu A, Dessi D, Rappelli P, Fiori PL (2018) Trichomonas vaginalis transports virulent mycoplasma hominis and transmits the infection to human cells after metronidazole treatment: a potential role in bacterial invasion of fetal membranes and amniotic fluid. J Pregnancy 2018:5037181. https://doi.org/10.1155/2018/5037181

Ton Nu PA, Rappelli P, Dessi D, Nguyen VQ, Fiori PL (2015) Kinetics of circulating antibody response to Trichomonas vaginalis: clinical and diagnostic implications. Sex Transm Infect 91 (8):561–563. https://doi.org/10.1136/sextrans-2014-051839

Trifonova RT, Pasicznyk JM, Fichorova RN (2006) Biocompatibility of solid-dosage forms of anti-human immunodeficiency virus type 1 microbicides with the human cervicovaginal mucosa modeled ex vivo. Antimicrob Agents Chemother 50(12):4005–4010. https://doi.org/10.1128/AAC.00588-06

Twu O, de Miguel N, Lustig G, Stevens GC, Vashisht AA, Wohlschlegel JA, Johnson PJ (2013) Trichomonas vaginalis exosomes deliver cargo to host cells and mediate hostratioparasite interactions. PLoS Pathog 9(7):e1003482. https://doi.org/10.1371/journal.ppat.1003482

Twu O, Dessi D, Vu A, Mercer F, Stevens GC, de Miguel N, Rappelli P, Cocco AR, Clubb RT, Fiori PL, Johnson PJ (2014) Trichomonas vaginalis homolog of macrophage migration inhibitory factor induces prostate cell growth, invasiveness, and inflammatory responses. Proc Natl Acad Sci U S A 111(22):8179–8184. https://doi.org/10.1073/pnas.1321884111

Tyagi RK, Tandel N, Deshpande R, Engelman RW, Patel SD, Tyagi P (2018) Humanized mice are instrumental to the study of Plasmodium falciparum infection. Front Immunol 9:2550. https://doi.org/10.3389/fimmu.2018.02550

Upcroft JA, Dunn LA, Wal T, Tabrizi S, Delgadillo-Correa MG, Johnson PJ, Garland S, Siba P, Upcroft P (2009) Metronidazole resistance in Trichomonas vaginalis from highland women in Papua New Guinea. Sex Health 6(4):334–338. https://doi.org/10.1071/SH09011

Van Gerwen, OT, & Muzny, CA. (2019). Recent advances in the epidemiology, diagnosis, and management of Trichomonas vaginalis infection. F1000Res 8. doi:https://doi.org/10.12688/f1000research.19972.1

van Niel G, D'Angelo G, Raposo G (2018) Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol 19(4):213–228. https://doi.org/10.1038/nrm.2017.125

Vancini RG, Pereira-Neves A, Borojevic R, Benchimol M (2008) Trichomonas vaginalis harboring Mycoplasma hominis increases cytopathogenicity in vitro. Eur J Clin Microbiol Infect Dis 27 (4):259–267. https://doi.org/10.1007/s10096-007-0422-1

Vasta GR (2012) Galectins as pattern recognition receptors: structure, function, and evolution. Adv Exp Med Biol 946:21–36. https://doi.org/10.1007/978-1-4614-0106-3_2

Vazquez-Carrillo LI, Q-GLI AR, Mendoza-Hernández G, González-Robles A, Carvajal-Gamez BI, Alvarez-Sánchez ME (2011) The effect of Zn2+ on prostatic cell cytotoxicity caused by Trichomonas vaginalis. J Integ OMICS 1(2):198–210

Vilela RC, Benchimol M (2013) IL-10 release by bovine epithelial cells cultured with Trichomonas vaginalis and Tritrichomonas foetus. Mem Inst Oswaldo Cruz 108(1):110–112. https://doi.org/10.1590/s0074-02762013000100018

Viscogliosi E, Brugerolle G (1994) Striated fibers in trichomonads: costa proteins represent a new class of proteins forming striated roots. Cell Motil Cytoskeleton 29(1):82–93. https://doi.org/10.1002/cm.970290108

Vrbanac A, Riestra AM, Coady A, Knight R, Nizet V, Patras KA (2018) The murine vaginal microbiota and its perturbation by the human pathogen group B Streptococcus. BMC Microbiol 18(1):197. https://doi.org/10.1186/s12866-018-1341-2

Walsh NC, Kenney LL, Jangalwe S, Aryee KE, Greiner DL, Brehm MA, Shultz LD (2017) Humanized mouse models of clinical disease. Annu Rev Pathol 12:187–215. https://doi.org/10.1146/annurev-pathol-052016-100332

Wang AL, Wang CC (1986) The double-stranded RNA in Trichomonas vaginalis may originate from virus-like particles. Proc Natl Acad Sci U S A 83(20):7956–7960. https://doi.org/10.1073/pnas.83.20.7956

Wang AL, Wang CC (1991) Viruses of parasitic protozoa. Parasitol Today 7(4):76–80. https://doi.org/10.1016/0169-4758(91)90198-w

Weber B, Mapeka TM, Maahlo MA, Hoosen AA (2003) Double stranded RNA virus in South African Trichomonas vaginalis isolates. J Clin Pathol 56(7):542–543. https://doi.org/10.1136/jcp.56.7.542

Wexler-Cohen Y, Stevens GC, Barnoy E, van der Bliek AM, Johnson PJ (2014) A dynamin-related protein contributes to Trichomonas vaginalis hydrogenosomal fission. FASEB J 28 (3):1113–1121. https://doi.org/10.1096/fj.13-235473

WHO (2018) Report on global sexually-transmitted infection surveillance

Wiggins R, Hicks SJ, Soothill PW, Millar MR, Corfield AP (2001) Mucinases and sialidases: their role in the pathogenesis of sexually transmitted infections in the female genital tract. Sex Transm Infect 77(6):402–408. https://doi.org/10.1136/sti.77.6.402

Wiringa AE, Ness RB, Darville T, Beigi RH, Haggerty CL (2019) Trichomonas vaginalis, endometritis and sequelae among women with clinically suspected pelvic inflammatory disease. Sex Transm Infect. https://doi.org/10.1136/sextrans-2019-054079

Woehle C, Kusdian G, Radine C, Graur D, Landan G, Gould SB (2014) The parasite Trichomonas vaginalis expresses thousands of pseudogenes and long non-coding RNAs independently from functional neighbouring genes. BMC Genomics 15:906. https://doi.org/10.1186/1471-2164-15-906

Wolf AJ, Underhill DM (2018) Peptidoglycan recognition by the innate immune system. Nat Rev Immunol 18(4):243–254. https://doi.org/10.1038/nri.2017.136

Xie YT, Gao JM, Wu YP, Tang P, Hide G, Lai DH, Lun ZR (2017) Recombinant alpha-actinin subunit antigens of Trichomonas vaginalis as potential vaccine candidates in protecting against trichomoniasis. Parasit Vectors 10(1):83. https://doi.org/10.1186/s13071-017-2009-8

Yang S, Zhao W, Wang H, Wang Y, Li J, Wu X (2018) Trichomonas vaginalis infection-associated risk of cervical cancer: a meta-analysis. Eur J Obstet Gynecol Reprod Biol 228:166–173. https://doi.org/10.1016/j.ejogrb.2018.06.031

Yap EH, Ho TH, Chan YC, Thong TW, Ng GC, Ho LC, Singh M (1995) Serum antibodies to Trichomonas vaginalis in invasive cervical cancer patients. Genitourin Med 71(6):402–404

Yarlett N, Martinez MP, Moharrami MA, Tachezy J (1996) The contribution of the arginine dihydrolase pathway to energy metabolism by Trichomonas vaginalis. Mol Biochem Parasitol 78(1-2):117–125

Yawata Y, Cordero OX, Menolascina F, Hehemann JH, Polz MF, Stocker R (2014) Competition-dispersal tradeoff ecologically differentiates recently speciated marine bacterioplankton populations. Proc Natl Acad Sci U S A 111(15):5622–5627. https://doi.org/10.1073/pnas.1318943111

Yellon SM (2017) Contributions to the dynamics of cervix remodeling prior to term and preterm birth. Biol Reprod 96(1):13–23. https://doi.org/10.1095/biolreprod.116.142844

Yildirim S, Yeoman CJ, Janga SC, Thomas SM, Ho M, Leigh SR, Primate Microbiome C, White BA, Wilson BA, Stumpf RM (2014a) Primate vaginal microbiomes exhibit species specificity without universal Lactobacillus dominance. ISME J 8(12):2431–2444. https://doi.org/10.1038/ismej.2014.90

Yildirim S, Yeoman CJ, Janga SC, Thomas SM, Ho M, Leigh SR, White BA, Wilson BA, Stumpf RM (2014b) Primate vaginal microbiomes exhibit species specificity without universal Lactobacillus dominance. ISME J 8(12):2431–2444. https://doi.org/10.1038/ismej.2014.90

Younes JA, Lievens E, Hummelen R, van der Westen R, Reid G, Petrova MI (2018) Women and their microbes: the unexpected friendship. Trends Microbiol 26(1):16–32. https://doi.org/10.1016/j.tim.2017.07.008

Yu J, Nagasu H, Murakami T, Hoang H, Broderick L, Hoffman HM, Horng T (2014) Inflammasome activation leads to Caspase-1-dependent mitochondrial damage and block of mitophagy. Proc Natl Acad Sci U S A 111(43):15514–15519. https://doi.org/10.1073/pnas.1414859111

Zariffard, MR, Harwani, S, Novak, RM, Graham, PJ, Ji, X, & Spear, GT. (2004). Trichomonas vaginalis infection activates cells through toll-like receptor 4. Clin Immunol, 111(1), 103-107. doi:https://doi.org/10.1016/j.clim.2003.12.008

Zuo Y, Riley DE, Krieger JN (1999) Flagellar duplication and migration during the Trichomonas vaginalis cell cycle. J Parasitol 85(2):203–207