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Clinical and Microbiological Aspects of *Trichomonas vaginalis*

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INTRODUCTION

Trichomonas vaginalis is a parasitic protozoan that is the cause of trichomoniasis, a sexually transmitted disease (STD) of worldwide importance. The disease encompasses a broad range of symptoms ranging from a state of severe inflammation and irritation with a frothy malodorous discharge to a relatively asymptomatic carrier state (48, 55, 309). Recent data have shown that the annual incidence of trichomoniasis is more than 170 million cases worldwide (311). More disturbing is the number of asymptomatic cases that are not treated. In North America alone, more than eight million new cases are reported yearly (311), with an estimated rate of asymptomatic cases as high as 50% (91, 309).

This disease has important medical, social, and economical implications. Women who are infected during pregnancy are predisposed to premature rupture of the placental membranes, premature labor, and low-birth-weight infants (70, 115, 214). Also linked to this disease are cervical cancer (110, 152, 315), atypical pelvic inflammatory disease (119), and infertility (111, 112).

As with other STDs, *T. vaginalis* infection can augment the predisposition of individuals to human immunodeficiency virus (HIV) infection (51, 171, 172). Laga et al. (171) found that the HIV seroconversion in female prostitutes was significantly associated with the occurrence of other nonulcerative STDs, such as trichomoniasis. It is thought that trichomoniasis may increase the transmission of HIV by causing local accumulations of HIV-infected or HIV-susceptible cells such as lymphocytes and macrophages (173).

GENERAL MORPHOLOGY

T. vaginalis is the most widely studied parasite of all the trichomonads. This urogenital pathogen varies in size and shape, with the average length and width being 10 and 7 μm, respectively (130). Physiochemical conditions do alter the appearance of the parasite. In axenic culture, the shape of the protozoan tends to be more uniform, i.e., pear shaped or oval (Fig. 1A) (25), but the parasite takes on a more amoeboid appearance when attached to vaginal epithelial cells (Fig. 1B and C) (25, 118).

T. vaginalis is a flagellated protozoan possessing five flagella, four of which are located at its anterior portion. The fifth

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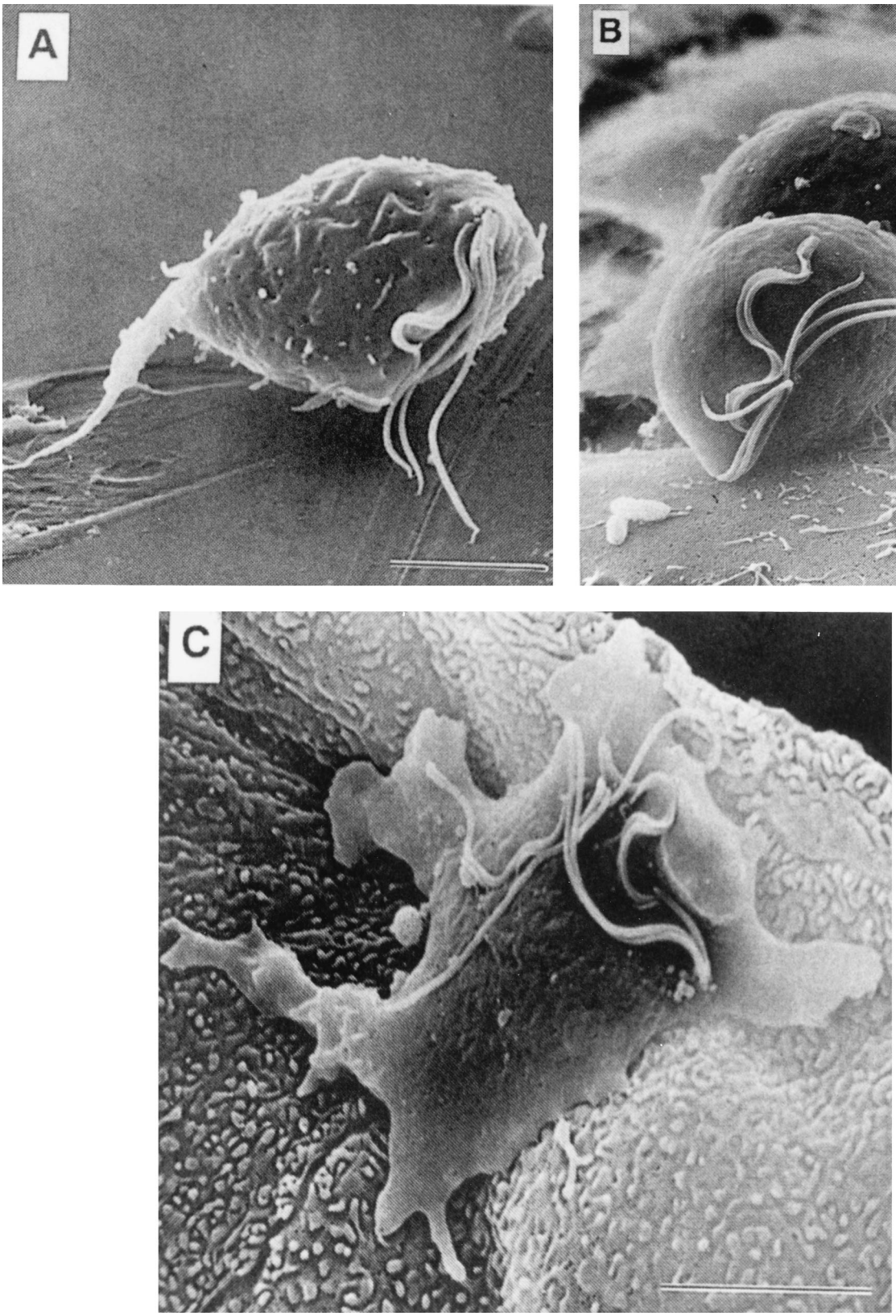

FIG. 1. (A) *T. vaginalis* parasite as seen in broth culture. The axostyle, undulating membrane, and flagella are clearly visible. (B) *T. vaginalis* on the surface of a vaginal epithelial cell prior to ameboid transformation. (C) Ameboid morphology of *T. vaginalis* as seen in cell culture. Note that the side opposite the undulating membrane adheres to the vaginal epithelial cell. Bars, 5 μm. Reprinted from reference 25 with permission of the publisher.

flagellum is incorporated within the undulating membrane of the parasite (Fig. 1A) (25, 300), which is supported by a slender noncontractile costa. The flagella and the undulating membrane give this parasite a characteristic quivering motility (130). Under unfavorable growth conditions, *T. vaginalis* can round up and internalize the flagella. Some believe these forms to be pseudocysts, but it is more likely that they are degenerate forms of *T. vaginalis*, since they have not been reported to give rise to normal motile forms (88, 127).

The cytoskeleton of *T. vaginalis* is composed of tubulin and actin fibers. Investigators have used monoclonal antibodies to the tubulin molecule and found that the axostylar tubulin reacted with both sheep and pig brain tubulin (54). The investigators also found that different types of tubulin are present within a trichomonad cell. Actin isolated from *T. vaginalis*, however, was shown to differ from that of pig and sheep skeletal muscle. *T. vaginalis* actin was observed to migrate more slowly than actin isolated from muscle when purified by anion-exchange chromatography, and it was found to have different peptide sequences as indicated by cleavage with proteolytic enzymes (54).

The nucleus in *T. vaginalis* is located at its anterior portion, and, as in other eukaryotes, it is surrounded by a porous nuclear envelope. A slender hyaline, rod-like structure, called an axostyle, commences at the nucleus and bisects the protozoan longitudinally. It protrudes through the posterior end of the parasite, terminating in a sharp point (130). This structure is thought to anchor the parasite to vaginal epithelial cells.

Granules are commonly seen in living organisms under light microscopy. These organelles are catalase negative, indicating that they are not peroxisomes (130). Because they produce molecular hydrogen, they were named hydrogenosomes and have been found to be important in metabolism (218). There are two sets of these granules: paracostal and paraxostylar. The latter set is arranged along the axostyle in three parallel rows, which is a distinguishing feature of *T. vaginalis*. Glycogen granules are also present in *T. vaginalis* and can be observed by transmission electron microscopy (50). *T. vaginalis* demonstrates hydrolase activity (27, 49) and contains lysosome-like structures such as phagosomes (233, 279–282).

REPRODUCTION AND LIFE CYCLE

Although cell division has been extensively described through the use of microscopy, the life cycle of *T. vaginalis* is still poorly understood. Like many other protozoan parasites, it is known to exist only as a trophozoite and lacks a cystic stage (127).

Several oversized round forms of the trichomonad are known to exist in dividing, growth phase culture: those without flagella, those with flagella and a dividing nucleus, and those with flagella and multiple nuclei. It was thought that these forms are not stages in the life cycle but, rather, that they arise during certain unfavorable conditions (127). However, recent evidence suggests that they may be developmental stages preceding the appearance of mononuclear flagellates (2). The round forms are morphologically different from the smaller, flagellated ovoid forms (2). Furthermore, they appear to divide by amitotic budding rather than by the mitotic division of ovoid cells (1, 2). It is not certain how these round forms fit into the development of the organism (2).

The small, ovoid flagellates generally reproduce by longitudinal binary fission, without the disappearance of the nuclear membrane (50). According to Brugerolle (50), this event begins with the duplication of selected locomotor organelles, which is followed by the development of two attractophores flanking either side of the nucleus, which become the poles for division. From the attractophores develop chromosomal microtubules, which extend toward and into the nucleus, attaching to the centromeres of the chromosomes. Also extended between the attractophores is an extranuclear spindle, called the paradesmose. This extranuclear spindle elongates, and the daughter cells separate. Each daughter cell then produces any missing organelles.

METABOLISM

T. vaginalis is a primitive eukaryotic organism. Although it is similar in many respects to other eukaryotes, it differs in its energy metabolism and shows remarkable similarity to primitive anaerobic bacteria. The hydrogenosomes (216) are analogous to the mitochondria of more advanced eukaryotes and carry out many of the same metabolic functions (218). The biochemistry and metabolism are well summarized by Müller (220).

Carbohydrate and Energy Metabolism

Being one of the most ancient eukaryotes, *T. vaginalis* has features that are common to anaerobic bacteria as well as higher eukaryotic organisms in terms of its carbohydrate and energy metabolism. Carbohydrate metabolism is described as being fermentative under both anaerobic and aerobic conditions because glucose is incompletely oxidized (194, 219). The metabolic products include acetate, lactate, malate, glycerol, CO_2, and, under anaerobic conditions, H_2 (181, 194, 271, 272).

Carbohydrate metabolism occurs in two compartments: the cytoplasm and an organelle called the hydrogenosome, which is analogous to the mitochondria of higher eukaryotes (133, 134, 146, 182, 183) and is found in a number of anaerobic parasitic protozoa (35, 61, 197). Within the cytoplasm, glucose is converted to phosphoenolpyruvate and subsequently to pyruvate via a classical Embden-Meyerhoff-Parnas pathway (181, 219, 221). Many of the enzymes in the pathway have been described (22, 304), and several steps produce energy via substrate-level phosphorylation. Glycerol is produced from dihydroxyacetone phosphate by glycerol-3-phosphate dehydrogenase and glycerol-3-phosphatase (59, 271). Lactate is also produced in the cytosol via the reduction of pyruvate by lactate dehydrogenase (183, 194). Pyruvate, which is generated through glycolysis, is then metabolized further in the hydrogenosome (271, 272).

The hydrogenosome, like the mitochondrion, is 0.5 to 1.0 μm in diameter and is surrounded by a double membrane (35, 37, 132). The hydrogenosomes are the site of fermentative oxidation of pyruvate (219, 221), and they produce ATP by substrate-level phosphorylation, produce hydrogen, process half of the carbohydrates of the cell, and contain homologous enzymes common to those found in both prokaryotes and eukaryotes (181, 197). Hydrogenosomes lack cristae and cytochromes, which are typically found in mitochondria (26). Furthermore, DNA is not present in the hydrogenosomes (146).

Biochemical studies of the hydrogenosome have revealed both similarities to and differences from mitochondria. Pyruvate:ferredoxin oxidoreductase, an enzyme not found in mitochondria, converts pyruvate to acetate (151). In this regard, *T. vaginalis* metabolism in the hydrogenosome is more closely related to that of the anaerobic bacteria (221). However, analysis of the ferredoxin protein in *T. vaginalis* shows it to be comparable to the [2Fe-2S] ferredoxins found in aerobic bacteria and in mitochondria (145) rather than in anaerobic bac-

teria. Another feature shared with mitochondria is the β-succinyl coenzyme A synthetase enzyme, which catalyzes ATP production by substrate-level phosphorylation (174).

Although the pathways in the two organelles may differ, they both carry out similar functions. This has led some investigators to believe that hydrogenosomes are modified or degenerate mitochondria (57). Others have suggested that hydrogenosomes and mitochondria arose from a common ancestral organelle rather than by the conversion of one from the other (113). Using sequence alignments of the 18S-like rRNA of various eukaryotes, Gunderson et al. (113) proposed that trichomonads branched off from the main line of the eukaryotic tree before true mitochondria arose. In support of this theory, Germot et al. have found a gene encoding a mitochondrial type of 70-kDa heat shock protein in *T. vaginalis* that has sequences which to date have been found only in mitochondrion HSP70 and proteobacterial DnaK (104). A third hypothesis suggests that hydrogenosomes originated through the endosymbiosis of an anaerobic bacterium and a primitive eukaryotic cell (216, 305). The similarities between the hydrogenosome and the anaerobic bacterium, in terms of anaerobic metabolism, support this theory.

Johnson and coworkers have done extensive research on the hydrogenosome and have characterized some of the genes and their respective products associated with this organelle, which include ferredoxin (145) and β-succinyl coenzyme A synthetase (174). As in mitochondria, *T. vaginalis* hydrogenosomal proteins have highly conserved amino-terminal leader sequences, which are cleaved upon entering the hydrogenosome. In the genes studied to date, these short leader sequences between 8 and 11 amino acids show a high degree of similarity to the longer leader sequences (20 to 80 amino acids) found in mitochondria. The leader sequences of the hydrogenosomal proteins are believed to be important in the targeting of specific proteins to the hydrogenosome (37, 174). Although the hydrogenosomes are characteristic of *T. vaginalis*, their functions may not be essential for the parasite, since trichomonads without the hydrogenosomes can be cultured in vitro (272).

Ter Kuile (286) and ter Kuile and Müller (288) have studied maltose utilization and transport. In most eubacteria and yeasts, maltose is transported into the cell and then hydrolyzed to glucose (257, 260). These investigators have demonstrated that as in the intestinal epithelial cells of vertebrates (259), maltose is cleaved on the cell surface of the parasite to glucose via α-glucosidase. Glucose is subsequently transported by the glucose transporter into the cytosol, where it is metabolized. This process, whereby the carbohydrate is metabolized on the membrane, does not seem to be advantageous for a unicellular organism, since glucose has been found to diffuse away from the parasite under culture conditions (288).

Chemostats have been used to study prokaryotes (287), but little work has been done with eukaryotic organisms (176). The use of chemostats has an advantage over the use of batch culture for growing organisms, since investigators can create conditions that closely approximate what occurs in vivo. Studies of *T. vaginalis* carbohydrate metabolism show that although the parasite is not exceedingly energy efficient, it is able to adapt its metabolism according to available carbon sources (286, 287). *T. vaginalis* has a high maintenance energy, expending up to half its carbon flow on maintaining internal homeostasis (287). Maintaining homeostasis is crucial for *T. vaginalis* since the vaginal environment is constantly changing with regard to pH, hormones, menses, and the nutrient supply.

Lipid Metabolism

T. vaginalis contains cholesterol, phosphatidylethanolamine, phosphatidylcholine, and sphingomyelin as its major phospholipids (29, 30). Lipid precursors fail to incorporate themselves into phospholipids in *T. vaginalis*, showing that the parasite is unable to synthesize fatty acids and sterols (29). Although lacking in the metabolic pathways necessary for phospholipid and fatty acid synthesis, *T. vaginalis* hydrolyzes the fatty acyl groups of phospholipids, triacylglycerols, and cholesterols and use these groups in the acylation of phospholipids (29, 30). Glycerolphospholipids are incorporated into most phospholipids, further suggesting that turnover of lipids in the membrane of the cell does occur (29). Nevertheless, many enzymes involved in the biosynthesis of complex phospholipids are lacking in *T. vaginalis*, and therefore the parasite must rely on exogenous sources of lipid moieties to survive (29, 30, 238).

Singh et al. have postulated that de novo glycolipid and glycophosphosphingolipid synthesis occurs in trichomonads (268). Glycoconjugates found on the surface of the parasite are important in its survival within the host microenvironment (267). A glycosphingolipid called TV_1 has been identified and found to contain inositol phosphoceramide. This appears to be a novel phospholipid since inositol phosphoceramide is normally substituted with ethanolamine. TV_1 may be an intermediate in the generation of membrane anchor proteins in *T. vaginalis* (69). Studies have shown that lipophosphoglycan-like glycoconjugates are also present on the cell surface. The role of these lipid moieties is not clear, although they may be involved in the survival of the parasite (267). Further study is required to determine the exact role that the phospholipids play in the host-parasite interrelationship.

Amino Acid Metabolism

Investigators have used high-pressure liquid chromatography to determine the amino acids present in the trichomonad cell (314). Carbohydrates are the preferred source of nutrients for *T. vaginalis*; however, under conditions where carbohydrates are limiting, amino acids have been shown to sustain trichomonad growth and survival (314). When grown in the absence of maltose, *T. vaginalis* consumes greater amounts of amino acids, especially arginine, threonine, and leucine which are used in energy generation (314). Under normal culture conditions, *T. vaginalis* consumes large amounts of arginine and somewhat smaller amounts of methionine for use in energy production (314).

Alanine (156, 255) and, more recently, glycine (314) have been found to be end products in the metabolism of glucose. Alanine and leucine constitute the major amino acids within the trichomonad cell (156, 255, 314). Valine, glutamate, phenylalanine, glycine, and proline levels are also elevated under standard conditions (156). In *T. vaginalis*, the intracellular and extracellular concentrations of amino acids are quite similar, implying that some form of equilibrium is being maintained between the free amino acids within the cell and its external environment (156).

Aminotransferases have not been extensively studied, but they have been shown to play a role in amino acid metabolism (192). Rowe and Lowe have purified an enzyme exhibiting dual activity: aspartate/aromatic amino acid:2-oxoglutarate aminotransferase (255). They have also found high activities of branched-chain and aromatic aminotransferases (255), which explains why these amino acids are found predominantly with *T. vaginalis*.

Nucleotide Metabolism

T. vaginalis lacks the ability to synthesize purines and pyrimidines and hence must resort to salvage pathways to generate nucleotides (120, 121). Purine salvage is mediated by nucleoside phosphorylases and kinases (213), whereas phosphoribosyltransferases and nucleoside kinases are able to recover pyrimidines (299). *T. vaginalis* requires adenine and guanine or their nucleosides for growth, in addition to thymidine, cytidine, uracil, and/or uridine (120, 184).

It is known that in cultured and animal cells, nucleosides enter the cells by passive diffusion through a single carrier (242). However, Harris et al. have postulated that *T. vaginalis* may in fact contain two separate carriers for nucleotide transport (116). One carrier is believed to accommodate adenosine and pyrimidine nucleosides, while the second shows a high affinity for uridine and a separate site for guanosine transport. The presence of two nucleoside carriers has also been found in another parasitic protist, *Leishmania donovani* (138).

NUTRITION, GROWTH REQUIREMENTS, AND CULTIVATION

Studies on the nutritional requirements of *T. vaginalis* came with the advent of the axenic culture technique. This organism evolved to survive in conjunction with host cells and various other members of the microbiological flora, and thus it was initially difficult to cultivate it independent of bacteria. Originally, an axenic culture was obtained by allowing the trichomonads to migrate down a tube, so that they swam free of other, nonmotile organisms (79, 106). Today, axenic culture is obtained through the use of antibiotics (81). The contributions of Diamond et al. (79, 81–83) and Linstead (184) to the development of media for trichomonads has enabled studies of the nutritional requirements of *T. vaginalis*.

T. vaginalis is an obligate parasite in that it lacks the ability to synthesize many macromolecules de novo, particularly purines, pyrimidines, and many lipids. These nutrients are acquired from the vaginal secretions or through phagocytosis of host and bacterial cells (119, 135). Culture media for *T. vaginalis* are thus required to include all the essential macromolecules, vitamins, and minerals. In particular, serum is essential for the growth of trichomonads, since it provides lipids, fatty acids, amino acids, and trace metals (81). Iron is also required, to maintain maximal levels of ferredoxin and pyruvate-ferredoxin oxidoreductase activity (109).

Diamond's TYM medium consists primarily of Trypticase, yeat, and maltose (79). The most popular medium for *T. vaginalis* currently is Diamond's TYI-S-33 medium (83), which is a nutrient broth of Trypticase, yeast extract, and iron, with the addition of fetal bovine serum and a vitamin 107-Tween 80 mixture (80).

Semidefined media and chemically defined media have been outlined by Linstead (184). In 1981, Linstead reasoned that since *T. vaginalis* is so intimately associated with its host cells, a modified cell culture medium might be suitable. Linstead's semidefined medium was developed from modified CMRL 1066 (Gibco) containing Tween 80. Often, however, the use of serum is undesirable because the proteins within it can interfere with enzymatic studies and can bind directly to the trichomonad (237). Linstead (184) therefore developed chemically defined media, DL7 and DL8, in which fetal calf serum was replaced with bovine serum albumin, cholesterol, and a mixture of fatty acids. Also supplied were 8 salts, various amino acids, and nucleic acid precursors, 7 carbohydrates, and 16 vitamins. Meysick and Garber (211) also developed a serum-free cell culture system using a McCoy cell monolayer. The presence of eukaryotic cells is necessary for cultures without serum.

In vitro, *T. vaginalis* grows optimally at a pH of 6.0 to 6.3 (81), although it does grow through a wide range of pHs, especially in the changing environment of the vagina (185).

CLINICAL MANIFESTATIONS

Trichomoniasis presents a wide variety of clinical patterns. The spectrum of clinical trichomoniasis in women ranges from the asymptomatic carrier state to flagrant vaginitis, with one-third of asymptomatic infected patients becoming symptomatic within 6 months (246). *T. vaginalis* principally infects the squamous epithelium in the genital tract. The infection, once established, persists for long periods in females but only for a short time in males. It is chiefly a disease of the reproductive years, and rarely are the clinical manifestations of the infection observed before menarche or after menopause. The incubation period is 4 to 28 days in about 50% of infected individuals. According to the severity of the infection, trichomoniasis may be classified as acute, chronic, or asymptomatic.

The clinical picture in the acute infection reveals diffuse vulvitis due to copious leukorrhea. The discharge is typically frothy, yellow or green, and mucopurulent (246). Small punctate hemorrhagic spots may be found on the vaginal and cervical mucosa. This speckled appearance has been referred to as a "strawberry appearance" and is observed in only 2% of patients (91). These signs and symptoms are cyclic and worsen around the time of menses.

In chronic infection, the predominant symptoms are mild, with pruritus and dyspareunia, while the vaginal secretion may be very scanty and mixed with mucus. This form of the disease is particularly important from the epidemiological point of view because these individuals are the major source of transmission of the parasite (227).

Up to 25 to 50% of infected women are asymptomatic and have a normal vaginal pH of 3.8 to 4.2 and a normal vaginal flora (270). Although there is a carrier form, 50% of these women will develop clinical symptoms during the subsequent 6 months.

Although vaginitis is the most common manifestation of *T. vaginalis* infection in women, Bartholin's gland is an occasional focus of infection. Other complications associated with trichomoniasis include adnexitis, pyosalpinx (258), endometritis (247), infertility (112), low birth weight (115), and cervical erosion (208). Trichomoniasis is also associated with increased HIV transmission (51, 171, 172).

Although *T. vaginalis* infection is regarded primarily as a disease of women, it also occurs in men. Trichomoniasis in men is largely asymptomatic, and these men are considered to be asymptomatic carriers of *T. vaginalis*. Urogenital trichomoniasis in men may be categorized into three groups: an asymptomatic carrier state, identified by investigation of sexual contacts of infected women; acute trichomoniasis, characterized by profuse purulent urethritis; and mild symptomatic disease, which is clinically indistinguishable from other causes of nongonococcal urethritis. Krieger (161) has shown that *T. vaginalis* is the cause of 11% of all cases of nongonococcal urethritis in males. The duration of infection is 10 days or less in most male patients. In symptomatic men, common complaints include scanty, clear to mucopurulent discharge, dysuria, and mild pruritus or burning sensation immediately after sexual intercourse (160). Complications associated with trichomoniasis include nongonococcal urethritis, prostatitis, balanoposthitis, epididymitis, urethral disease, and infertility (124, 159, 198, 293).

Trichomoniasis is seen much less frequently in the male population and is associated with fewer than 5% of urethral infections. Most cases (14 to 60%) are associated with known infected female partners (28, 303, 308).

DIAGNOSIS

Clinical Presentation

The classic symptoms associated with the clinical diagnosis of *T. vaginalis* include a yellowish-green frothy discharge, pruritus, dysuria, dyspareunia, and the "strawberry" cervix, which is characterized by punctate hemorrhagic lesions (85). However, diagnosis cannot be readily made solely on the basis of clinical presentation for several reasons: (i) the clinical symptoms may be synonymous with those of other STDs (143, 204, 248, 309), (ii) the classic "strawberry" cervix is seen in approximately only 2% of patients, and (iii) the frothy discharge is seen in only 12% of women with *T. vaginalis* (91). In 1980, Fouts and Kraus (91) demonstrated that if these classic features are used alone in the diagnosis of trichomoniasis, 88% of infected women will not be diagnosed and 29% of uninfected women will be falsely indicated as having infection (91). The data suggest that clinical manifestations are not reliable diagnostic parameters and that laboratory investigations are necessary for the accurate diagnosis of trichomoniasis. Accurate diagnosis is essential, since it will lead to appropriate treatment and will facilitate the control of the spread of *T. vaginalis* infection.

Microscopic and Culture Techniques

Diagnosis of trichomoniasis has traditionally depended on the microscopic observation of motile protozoa in vaginal or cervical secretions, a procedure first described by Donné in 1836 (85). Trichomonads can be differentiated on the basis of their characteristic motility. The sensitivity of this technique varies from as low as 38% to as high as 82% (136, 199, 203). Although this method is certainly the most cost-effective diagnostic test, it is far from optimal in terms of its reliability because it has low sensitivity. This may be due to the loss of distinctive motility after the protozoan has been removed from body temperature.

The broth culture method is the "gold standard" for the diagnosis of trichomoniasis because it is simple to interpret and requires as few as 300 to 500 trichomonads/ml of inoculum (101) to initiate growth in culture. However, there are inherent limitations to culture diagnosis (91). An incubation period of 2 to 7 days is usually necessary to identify *T. vaginalis* in cultures, during which time infected patients may continue to transmit the infection (215). Also, no culture systems are widely available to clinicians. To improve the acceptability of diagnosis by culture, a plastic envelope method was devised, which permits both immediate examination and culture in one self-contained system (31). The results are comparable to those of wet-preparation and culture techniques (31). Similar to the plastic envelope method the InPouch system is a two-chambered bag that allows one to perform an immediate wet-mount by microscopic examination through the bag, as well as a culture. Levi et al. (180) showed that the InPouch system is at least as sensitive as Diamond's modified medium for the detection of *T. vaginalis*. Borchardt et al. (41) showed that this system is significantly more sensitive than either Diamond's modified medium or Trichosel medium.

The cell culture technique uses a variety of cell lines to recover *T. vaginalis* from clinical specimens (118, 164, 240). Garber et al. (101) used McCoy cells for the cultivation of *T. vaginalis* from clinical specimens and showed this method to be superior to the broth culture and wet-mount preparation since it was able to detect *T. vaginalis* at a concentration as low as 3 organisms/ml. However, cell culture is not routinely performed, because it is expensive and not convenient for rapid diagnosis.

Because cultivation methods are relatively slow and wet-mount preparation lacks sensitivity, the staining of parasites in fixed and unfixed smears was introduced. Staining techniques such as acridine orange (92), Leishman (179), periodic acid-Schiff (250), and Fontana (224) have been introduced to improve the sensitivity of direct microscopy. Papanicolaou (Pap) staining holds considerable appeal in the diagnosis of trichomoniasis because it is routinely used in gynecologic screening for cytologic abnormalities, particularly in populations with a high prevalence of STD (38, 179, 269). Perl (235), however, reported a 48.4% error in diagnosis due to false-positive and false-negative findings when Pap smears were used as the sole criterion for diagnosis and treatment of *T. vaginalis* infection. Staining techniques have their limitations since *T. vaginalis* does not always appear in its typical pear-shaped form with flagella. It often appears as rounded forms resembling polymorphonuclear leukocytes, and occasionally the typical morphologic characteristics are lost during fixation and staining, making the etiologic identification difficult (235).

The limitations of culture and microscopy methods for the detection of *T. vaginalis* prompted the advance of the more sophisticated methods which can detect antigen, antibody, or nucleic acids in urethral or vaginal exudate.

Antibody-Based Techniques

There are an estimated eight serotypes observed in *T. vaginalis* (4). However, by immunoblot analysis, a wide variety of antigenic markers are seen (100). Various techniques including agglutination, complement fixation, indirect hemagglutination, gel diffusion, fluorescent antibody, and enzyme-linked immunosorbent assay have been used to demonstrate the presence of antitrichomonal antibodies (142, 201, 202, 265, 284). However, the serum or local antibody response to a pathogen depends on several factors, such as the nature of the antigen or pathogen, its live or inactivated form, its local concentration, and the frequency and length of immune system stimulation. It has several inherent disadvantages. In some instances, an antibody response is not observed either because the system is too insensitive to detect low levels of specific antibodies or because the serum humoral response has not yet been elicited. Since trichomonal antibodies may persist for a long time after treatment, current and past infections cannot be distinguished.

Direct detection of *T. vaginalis* antigens in clinical specimens by using monoclonal antibodies holds promise as a rapid method in the diagnosis of trichomoniasis. Krieger et al. (162) obtained two broadly reactive monoclonal antibodies which identified all 88 strains of *T. vaginalis* obtained from diverse geographic areas of North America. Monoclonal antibodies to immunogens such as the 62- and 65-kDa proteins for the detection of *T. vaginalis* from clinical specimens gave similar results to those of wet-mount preparations (186). Furthermore, the use of monoclonal antibodies to proteins such as cell-detaching factor (CDF; 200 kDa) and cysteine protease (60 kDa), which are immunogens observed in all the isolates of *T. vaginalis*, could provide an alternative method for the detection of trichomoniasis. Trichomonas Direct Enzyme Immunoassay and Fluorescent Direct Immunoassay (California Integrated Diagnostics, Benicia, Calif.), which use peroxidase-

and fluorochrome-labeled cocktails of monoclonal antibodies to various *T. vaginalis* structures, were as sensitive and specific as culture techniques (291). Also, the results can be obtained in 1 h, allowing both diagnosis and administration of treatment in a single patient visit.

DNA Techniques

Recombinant DNA techniques have been increasingly used in clinical laboratories to improve the specificity and sensitivity of *T. vaginalis* diagnosis. The use of PCR methods helps detect nonviable organisms and also has the ability to detect cells and target sequences in clinical samples that have undergone fixation or partial degradation (249). The commercially available Affirm VP system (MicroProbe Corp, Bothwell, Wash.) uses synthetic oligonucleotide probes for the detection of both *Gardnerella vaginalis* and *T. vaginalis* from a single vaginal swab (47). The technique was found to be superior to wet-mount preparation. However, false-negative results were encountered when this technique was compared with culture technique (80% sensitive compared with culture-positive samples) (47).

The dot-blot hybridization technique, which uses a 2.3-kb *T. vaginalis* DNA fragment as a probe, can detect *T. vaginalis* DNA from vaginal exudate. However, because of the instability of the probe and the special care needed in the handling and disposal of the radioactive material, the technique has significant drawbacks (256). These limitations could be overcome by using the 2.3-kb fluorescence-labeled DNA probe for identification of *T. vaginalis* by the DNA in situ hybridization technique. The usefulness of these techniques for identifying asymptomatic carriers needs to be evaluated.

TREATMENT

Until 1959, topical vaginal preparations available against trichomoniasis provided some symptomatic relief but were ineffective as cures (190). These local treatments did not penetrate the vaginal epithelium, urethra, Skene's glands, and Bartholin's glands, which harbor the organism (190). Even if females were treated successfully, there was no treatment for the male partners, and so reinfection could quickly occur. More modern vaginal preparations including clotrimazole, povidone-iodine, and nonoxynol-9 are also palliative, and their effectiveness as a cure is either unacceptable or undefined (190).

In 1959, a nitroimidazole derivative of a *Streptomyces* antibiotic, azomycin, was found to be highly effective in the systemic treatment of trichomoniasis (66). This derivative was α,β-hydroxyethyl-2-methyl-5-nitroimidazole, commonly referred to as metronidazole and marketed under the trade name Flagyl. Other nitroimidazoles, although unavailable in North America, are also approved for clinical use in other parts of the world. These include tinidazole (278), ornidazole (93), secnidazole (296), flunidazole (234), nimorazole (117), and carnidazole (60).

These nitroimidazoles are not themselves cytocidal against *T. vaginalis*, but their metabolic products are (119). Metronidazole enters the cell through diffusion (222) and is activated in the hydrogenosomes of *T. vaginalis* (217). Here, the nitro group of the drug is reduced anaerobically by pyruvate-ferredoxin oxidoreductase (217). This results in cytotoxic nitro radical-ion intermediates that break the DNA strands (292). The response is rapid; cell division and motility cease within 1 h and cell death occurs within 8 h as seen in cell culture (229).

Today, the standard treatment for trichomoniasis is 250 mg of metronidazole, given orally, three times a day for 7 days, or in a single 2-g dose. Both the infected patient and sexual partner, whether symptomatic or asymptomatic, should be treated to prevent reinfection. Both regimens are equally effective (114, 294). The success rate ranges from 82 to 88% and is almost 95% when the sexual partner is treated simultaneously (119). The single 2-g dose is preferred, since less total drug is required, patient compliance is better, and there are fewer side effects (114, 189). Metronidazole is well absorbed through most mucous membranes, and the efficacy of drug delivery to the vaginal epithelium via oral routes is assumed, but not well studied, in women who do not respond to treatment.

Metronidazole has a low risk of causing birth defects in pregnant women (253) and a low risk of causing cancer (32). It does cross the placental barrier and therefore is not indicated for the treatment of trichomoniasis in women who are in the first trimester of pregnancy (190), although human birth defects have not been directly associated with its use. Pregnant women who have severe symptoms can be treated with 100-mg clotrimazole suppositories intravaginally at bedtime for 14 nights. A cure rate of 50% is achieved with this method. If further systemic treatment is necessary, it is delayed at least until the second or third trimester, when a single 2-g dose of metronidazole is given (190). The treatment for lactating mothers is a single 2-g dose followed by interruption of breast feeding for at least 24 h (189). Neonatal trichomoniasis is dependent on maternal estrogen levels, which begin to wane after the third to sixth week of life. After this time, the infection may disappear (189). Treatment of neonates should therefore be postponed until the sixth to eighth week of age and then given only if the infant is symptomatic (189).

Although the cure rate is excellent, treatment failure is problematic. Most often, treatment failure is due to noncompliance or reinfection. Other proposed reasons for treatment failure are low zinc concentrations in serum (307), poor absorption of the drug (149), ineffective delivery of the drug to the vaginal area, or inactivation of the drug by bacteria within the vagina (86, 137, 205). Lumsden et al. (193) considered these mechanisms to be unlikely and suggest that true resistance of *T. vaginalis* to the drug is the cause of many treatment failures. The many published case reports of clinically resistant trichomoniasis undoubtedly show that resistance of *T. vaginalis* to metronidazole is on the rise. In 1989, the Centers for Disease Control estimated that 5% of all *T. vaginalis* patient isolates had some level of resistance to metronidazole (225).

Resistance can be due to several mutational changes and can affect both aerobic and anaerobic mechanisms of metabolism. The mechanisms of metronidazole resistance in *T. vaginalis* strains have been studied in vitro (170, 244, 283). In trichomonads resistant by aerobic mechanisms, the transcription of the ferredoxin gene is reduced, thereby decreasing the ability of the cell to activate the drug (244). In anaerobic resistance, the activities of pyruvate-ferredoxin oxidoreductase and hydrogenase are decreased or nonexistent (170).

T. vaginalis should be tested for susceptibility to metronidazole, since this can explain clinical failure when compliance is not an issue and may dictate the next course of treatment. In vitro susceptibility tests for *T. vaginalis* are best reflected by aerobic cultivation, since there is poor discrimination of susceptibility in anaerobic culture. Unfortunately, the minimum lethal concentrations in susceptibility assays do not reflect the drug concentrations in serum needed for cure (191), but they can provide assistance in estimating the dosage of drug likely to be curative (190, 223).

Refractory cases, for which a second standard treatment is not curative, are treated with higher doses of metronidazole

TABLE 1. Recovery of *T. vaginalis* from mouse vaginal washes[e]

Group[a]	Dose or treatment[a]	No. of mice with *T. vaginalis* on the indicated day of culture/total no. inoculated			
		+7	+14	+21	+28
1	4.5×10^5 organisms/ml ($n = 26$)	12/26	10/25[b]	8/25[b,c]	7/25[b,c]
2	9×10^6 organisms/ml ($n = 26$)	6/25[b,c]	5/25[b,c]	3/24[b,c,d]	1/24[b,c,d]
3	1×10^8 organisms/ml ($n = 26$)	6/25[b,c]	4/23[b,c]	1/21[b,c,d]	0/21[b,c,d]
4	PBS ($n = 18$)	16/17[b]	13/16[b]	9/15[b]	8/14[b]
5	No immunization ($n = 18$)	14/16[b]	12/16[b]	10/15[b]	7/15[b]
6	No treatment (negative control) ($n = 6$)	0/6	0/6	0/6	0/6

[a] All mice in groups 1 to 5 received 0.05 ml of estradiol valerate subcutaneously on days −9 and −2, vaginal inoculation with 10^{10} *L. acidophilus* organisms per ml on days −7 and −6, and vaginal infection with 2.5×10^7 *T. vaginalis* organisms per ml. Groups 1 to 4 received subcutaneous inoculation of *T. vaginalis* on days −56 (in Freund's complete adjuvant) and −28 (in Freund's incomplete adjuvant) in the doses indicated. PBS, phosphate-buffered saline. Group 6 received none of the above manipulations.

[b] Total does not equal *n* because of unrelated death of mice.

[c] Statistically significant compared with group 4 value.

[d] Statistically significant compared with group 1 value.

[e] Reprinted from reference 3 with permission of the publisher.

(often double) for an extended period; this is effective only 80% of the time (225). For each level of resistance (marginal, low, moderate, or high), there is a recommended treatment regimen (190). Ahmed-Jushuf et al. (5) also described a treatment protocol for refractory cases. Organisms with high levels of resistance are difficult to treat and may require very high, toxic amounts of drug, often administered in an oral and vaginal combination or sometimes intravenously to reduce side effects. When this fails, physicians must experiment with different variations of existing treatments and protocols.

Clearly, new antitrichomonal agents are needed to treat resistant organisms. Although there are many other nitroimidazoles, only metronidazole is available in North America. Furthermore, all the nitroimidazoles have similar modes of antimicrobial activity (193), and so resistance to metronidazole often includes resistance to the other nitroimidazoles (225). It is therefore wise to consider other potential drugs for the treatment of trichomoniasis, including furazolidone (Trichofuran) (225), mebendazole (148), butoconazole (42), benzoizothiazolinon (316), and gynalgin (266).

VACCINATION AND IMMUNITY

Recently, Abraham et al. (3) was able to induce immunity to *T. vaginalis* in the mouse model, which may lead to the development of a vaccine. Immunity has been difficult to produce in vivo, since in humans, repeated infections with *T. vaginalis* do not confer immune protection (4, 126). Despite this, antibodies can be found in the serum (64, 100, 265, 275, 312) and vaginal secretions (196, 275, 277, 313) of infected individuals and a cell-mediated immune response is also invoked (175, 313).

To date, only one vaccine had been produced against *T. vaginalis*. The Solco Trichovac vaccine was prepared from inactive lactobacilli and was thought to work by inducing antibodies to abnormal lactobacilli and *T. vaginalis* without adversely affecting the growth of normal lactobacilli in the vagina (108). However, a lack of antigenic similarity between this vaccine and *T. vaginalis* was shown (7, 108), which makes this cross-reaction hypothesis unlikely. Clinical trials of Solco Trichovac have yielded inconclusive data (270).

In the study by Abraham et al. (3), whole, live trichomonads at different concentrations were injected subcutaneously into mice, first with Freund's complete adjuvant and then in a booster dose with the trichomonads and Freund's incomplete adjuvant. The mice were given estrogen and inoculated intravaginally with *Lactobacillus acidophilus* to simulate the conditions in the human vagina (206, 212); they were then inoculated intravaginally with *T. vaginalis*. Immunized mice had significantly less intravaginal infection and had elevated antibody levels in the serum and vagina compared with the sham-inoculated and naive control groups (Table 1). Mice that had been infected vaginally, treated, and reinfected vaginally were not protected and did not mount an immune response. This suggests that antigen presentation may be crucial for developing protective immunity. This work provides new hope for the goal of vaccine development, and further studies are warranted.

EPIDEMIOLOGY AND MODES OF TRANSMISSION

Trichomonal infection has been encountered in every continent and climate and with no seasonal variability. It has a cosmopolitan distribution and has been identified in all racial groups and socioeconomic strata. The estimated incidence is more than 170 million cases worldwide (311), and at least 2 to 3 million symptomatic infections occur annually among sexually active women in the United States (48, 310). The incidence of trichomoniasis is as high as 56% in patients attending STD clinics (290). This rate depends on many factors including age, sexual activity, number of sexual partners, other STDs, sexual customs, phase of the menstrual cycle, techniques of examination, specimen collection, and laboratory technique.

Humans are the only natural host for *T. vaginalis*. The trophozoite is transmitted from one person to another, usually by sexual intercourse. Four points support the belief that *T. vaginalis* is transmitted sexually. The most important evidence is the high rate of infection of the urethra and/or prostate in the male partners of infected females. In such cases, recurrent trichomonal vaginitis was cured only after the parasites had been eradicated from the genital tract of the patients' consorts. *T. vaginalis* is observed more frequently in females attending STD clinics and also in prostitutes than in postmenopausal women and virgins. Finally, the flagellates die outside the human body unless they are protected from drying (55, 150, 158, 306). Nonsexual transmission has been observed in cases such as by contaminated douche nozzles, specula, or toilet seats through which trichomonads may find their way into the vagina (306). However, such cases are rare. Live *T. vaginalis* has been found in urine and in semen after several hours of exposure to air and in swimming pool water (56).

Newborn infants of mothers infected by *T. vaginalis* have on

occasion acquired a *T. vaginalis* urinary tract or vaginal infection. The organisms were acquired by 2 to 17% of female neonates of infected women (21).

In the United States, black women have higher rates of trichomoniasis than white women, and socioeconomic factors such as a lower level of education appear to be associated with a higher prevalence rate of trichomoniasis (55, 143). Oral contraceptive use has been associated with a lower infection rate, and the infection is more prevalent in the age group of 20 to 45 years (39, 44), which is older than for most other STDs. Because of the wide spectrum of clinical disease, control of this infection relies on the screening of women and their partners and the appropriate treatment of infected individuals to prevent the continued spread of the disease.

EXPERIMENTAL MODELS AND VIRULENCE ASSAYS

Many animals have been proposed as models of *T. vaginalis* infection and have been discussed in great detail by Honigberg (125) and Kulda (168). These include monkeys, hamsters, guinea pigs, rats, mice, cattle (65), and dogs (73, 165). Most of these animals have generally not met the expectations of a suitable model due to the lack of ability to sustain genital infection, lack of symptomatic disease, poor immune response, presence of contaminating indigenous trichomonads of the gut, or costs and limitations involved in the housing and care of a particular species. Furthermore, few studies have addressed infection of males (73, 144, 165, 252). This lack of a good model has severely limited our ability to conduct standardized, controlled research on transmission, pathogenesis, immune response, and drug and vaccine development. Clearly, animal models which accurately imitate trichomoniasis need better refinement to achieve a consistent, long-lasting genital infection.

Two recent models seem to be encouraging. Vaginal infection of the squirrel monkey seems to result in symptomatic disease, for periods of up to 3 months, with horizontal transmission (103, 273). On the other hand, the immune response seems weak and the presence of indigenous trichomonads which require eradication is worrisome to some researchers (123). Recent studies have been too small to adequately assess the appropriateness of the squirrel monkey as a model, and more research is required.

The mouse has been by far the most popular animal used in experimental models of *T. vaginalis* infection. There has been some variability in terms of the length of infection, although sexual transmission has been achieved and symptoms have been reported (168). Intravaginal infection has been successful only after pre-estrogenization. This step, which potentiates infection by inducing estrus and by enhancing the glycogen levels within the vagina, is required only to establish the infection (53) but is not required for maintenance (295). Colonization of the vagina with either *Candida albicans* or *L. acidophilus* is thought to better parallel the human conditions and has resulted in increased infection rates (206, 209). The mouse model of McGrory and Garber (206), in which the vagina is precolonized with lactobacilli, sustained consistent infection for at least 4 weeks. However, Corbeil (65) recently pointed out that because *T. vaginalis* is not a naturally occurring organism in mice, it is uncertain whether the virulence factors are species specific or what effect estrogenization has on the murine immune system. Corbeil reasoned that because *T. vaginalis* and *Tritrichomonas foetus* share many virulence factors, we can learn a great deal from the study of *T. foetus* infection in cattle, which is naturally occurring.

Other murine models, such as the intraperitoneal assay of Teras and Roigas (285) and Cavier et al. (58) and the subcutaneous assay of Honigberg et al. (131), have aided the study of virulence. Intraperitoneal injection of trichomonads produces visceral (especially pancreatic and hepatic) necrosis, whose extent is proportional to the level of virulence of the inoculated strain, which can result in death. Subcutaneous inoculation of *T. vaginalis* results in the development of a localized abscess at the injection site. The volume of the lesion can be used to estimate the level of virulence. While some studies dispute the existence of a correlation between these assays and virulence (141), Kulda (168) has compared these virulence assays and maintains that although some irregularities exist, there is a complementary correlation between each virulence assay and clinical disease, provided that the protocols are strictly followed. The subcutaneous assay may be more useful in differentiating virulent from avirulent trichomonads, but its correlation with clinical disease is poor (96). Increasingly, in vitro virulence assays are being performed either alongside or in place of animal model virulence assays. Of particular importance are assays for hemolytic activity (163), adherence in cell culture, and cytotoxicity in cell culture (20, 45, 94, 167). Data from virulence assays must be interpreted with caution, however, since they do not always correlate with or are not always relevant to clinical phenomena (46, 166).

PATHOGENESIS

Although *T. vaginalis* is the most intensely studied trichomonad and is the world's most common cause of nonviral STDs, the exact mechanism of its pathogenesis has not been clearly elucidated. Honigberg's research into trichomonal pathogenesis provided much of the groundwork for later researchers. While searching for a virulence assay (62, 131), Honigberg and coworkers used microscopy and biochemical analysis to define trichomonad structure and function (89, 132, 169, 300–302) as well as many behavioral and cytochemical interactions between the parasite and the host cell (129, 262–264). Modern research has focused on the initial events required to establish infection. Many mechanisms are thought to be involved and include cell-to-cell adhesion (10, 14, 18, 24, 52, 67, 68, 107), hemolysis (72, 90, 163, 178), and the excretion of soluble factors such as extracellular proteinases (23, 97, 195) and CDF (98, 240). The interaction of *T. vaginalis* with the members of the resident flora of the vagina may be an important factor as well (207), and, like many other protozoans, *T. vaginalis* has demonstrated many mechanisms which are used to evade the host immune system (9, 11, 20, 78, 154, 243). The host-parasite relationship is very complex, and the broad range of clinical symptoms cannot likely be attributed to a single pathogenic mechanism. All clinical isolates of *T. vaginalis* appear to be capable of infection and disease production.

The cell surface of the trichomonad plays a major role in adhesion, host-parasite interaction, and nutrient acquisition, and the proteins and glycoproteins displayed on the surface have functions in this regard. Molecular analysis of these membrane macromolecules and virulence factors has really just begun.

Adherence and Adhesins

Adhesion of trichomonads to the epithelial cells in the vaginal environment is a critical step in the pathogenesis of the parasite (13, 19, 118, 164). Attachment to cells is time, temperature, and pH dependent. *T. vaginalis* appears more inclined to parasitize vaginal epithelial cell lines than other cell

types in vitro. This is not surprising since epithelial cells are likely to be the principal cell type with which the parasite would interact in vivo (13). The surface of the trichomonad cell is a mosaic of adhesins, receptors to host extracellular matrix proteins, and carbohydrates, which provide the basis for ligand-receptor binding (40).

The adhesion of the parasite to the epithelial cell seems to be mediated by four adhesion proteins: AP65, AP51, AP33, and AP23 (24, 87), which act in a specific receptor-ligand fashion (14, 24, 25). AP65 is encoded by at least three genes in a multiple-gene family (18, 232) which are curiously similar to the genes encoding malic enzyme (87, 232). The adhesins are alternately expressed on the surface with a highly immunogenic glycoprotein, P270 (8, 9, 14); the biological significance of this alternating phenotypic variation is discussed further in the section on immune system evasion (below). Gene expression of the four adhesins is coordinately upregulated at the transcriptional level by iron (18, 177). Contact-initiated ameboid transformation, which is marked by the production of pseudopodia followed by the upregulation of adhesin synthesis, suggests evidence for a sophisticated signal transduction system (25). Adhesion seems to also require the presence of cysteine proteinases (CPs) (23).

It has been observed that the side opposite the undulating membrane and the recurrent flagellum of the parasite attaches itself to the epithelial cells (13), and the microfilaments become concentrated in the parasite on the side that is in contact with the vaginal epithelium (245).

While the adhesins are concentrated on the side opposite the undulating membrane, laminin-binding proteins are ubiquitous on the entire surface of *T. vaginalis* (68). Laminin, a glycoprotein localized in the basement membrane of the epithelium, promotes cell adhesion, differentiation, shape, and motility in normal cells, and it has been shown to have chemotactic properties (68). *T. vaginalis* was observed adhering to laminin-coated plastic (68) and endocytosing laminin-covered polystyrene particles (34). Similar laminin receptors have also been found on macrophages, bacteria, and cancer cells (188), but their role in *T. vaginalis* pathogenesis remains undefined.

Similarly, *T. vaginalis* has receptors for another extracellular matrix adhesion glycoprotein, fibronectin, which is secreted in both the basement membrane and serum. Although trichomonads can become coated with host fibronectin (and other serum proteins) (236), it is unclear whether fibronectin receptors function in nutrient acquisition (236, 237), adherence (10, 239, 289), or a combination of the two (107).

Lectin-binding carbohydrates on the surface of the trichomonad cell were characterized by the group of Honigberg (62, 301, 302), after Cappuccinelli et al. (52) reported their role in adherence to glass. The presence of surface carbohydrates (D-lactose and *N*-acetyl-D-glucosamine) appears to be correlated with virulence (62, 157, 301). Surface saccharides seem to be involved in *T. vaginalis* hemolysis of erythrocytes (76) and phagocytosis of the target cells (36) and may be associated with drug susceptibility (84). On the other hand, endogenous lectins on the surface of the trichomonad cell may be important in adhesion (200, 254).

Neuraminidase seems to be excreted both on the surface and in the culture media (67, 210). Cleavage of sialic acid on the surface of host cells may be important for adhesion (40, 210), but sialic acid present on the surface of *T. vaginalis* does not seem to be involved (40).

Adherence, however, does not correlate directly with virulence, since virulent strains isolated from symptomatic patients exhibited wide differences in their ability to adhere to host cells (166). This illustrates the complex host-parasite relationships of *T. vaginalis*.

Hemolysis

The vaginal mucosa may be a poor nutritional milieu for microbes. Since the ability to synthesize lipids is lacking in *T. vaginalis*, erythrocytes may be a prime source of fatty acids that are needed by the parasite. In addition to lipids, iron is an important nutrient for *T. vaginalis* and may also be acquired via the lysis of erythrocytes (178).

Metabolically active parasites are necessary for lysis of erythrocytes. CP inhibitors greatly reduced erythrocyte lysis, which suggests that CPs may be a lytic factor involved in hemolysis (72). Hemolysis in vitro is greatest at the normal vaginal pH of 4.5, suggesting that this parasite characteristic occurs within the vaginal microenvironment (90).

This lysis of the erythrocytes appears to be mediated by protein receptors on the surfaces of both erythrocytes and parasites (90), and empirical evidence suggests that perforin-like proteins may be involved. Five adhesin molecules have been identified, three of them identical to the ones that mediate adherence to epithelial cells (24, 90). Hemolysis seems to occur in three steps. A specific ligand-receptor interaction allows the trichomonad to attach itself to the erythrocyte. This is followed by the release of perforin-like proteins (possibly CPs), which form pores in the erythrocyte membrane. Finally, *T. vaginalis* detaches itself from the cell and cell lysis occurs. Unlike its behavior with epithelial cells, *T. vaginalis* has been observed to phagocytize erythrocytes (90). Hemolytic activity appears to be correlated with virulence (163).

Proteinases

Characteristics of proteinases have been well summarized by North (230, 231), and extensive work has been done in isolating and purifying the proteinases of *T. vaginalis* (139, 187). *T. vaginalis* has between 11 and 23 distinct CP activities, most of which are lysosomal (23, 95, 97, 226, 243). The CPs of *T. vaginalis* are by far the most abundant of the parasitic protozoa. It is not surprising that these enzymes play a role in the pathogenesis of the parasite. Irvine et al. have used various CP inhibitors in an attempt to determine which CPs are essential to parasite survival (140).

CPs have been implicated as probable lytic factors in the hemolysis of erythrocytes. In addition, CP activity is required for the adherence of *T. vaginalis* to epithelial cells (23). Pretreating trichomonads with *N*α-*p*-tosyl-L-lysine chloromethyl ketone HCl (TLCK), a CP inhibitor, caused a marked decline in their ability to adhere to epithelial cells. When CP was added to TLCK-treated cells, their ability to attach themselves to the host was restored (23). This indicates that the action of proteinase on the surface of the parasite is a prerequisite for host attachment.

T. vaginalis CPs also have the ability to degrade host immunoglobulins G and A (IgG and IgA) (243), both of which are present in the vagina. The biological implications of this activity are discussed in the section on immune system evasion, below.

Contact-Independent Mechanisms of Pathogenicity and Cell-Detaching Factor

Although contact-dependent mechanisms play a significant role in the pathogenesis of *T. vaginalis*, contact-independent mechanisms are also involved. The first to report on contact-independent mechanisms was Hogue (122), who noted that

cell-free filtrates had similar adverse effects on cell culture to those of the organism itself. The idea that some soluble cytotoxin may play a role in the pathogenic effects has also been proposed by others (12, 89, 125, 128, 228). Hemolysis and cytotoxicity, for example, cannot be explained solely by the contact-dependent mechanisms, since these effects can be seen in the absence of cell-to-cell contact (94, 241). While pH and acidic metabolites can be partly responsible for these effects (94, 241), the organism has been shown to produce other factors which cause cytopathic effect.

It has been shown that a cell-free product of *T. vaginalis*, CDF, causes cytopathic effects in cell culture (98, 240). When the cell-free filtrate of a *T. vaginalis* culture is applied in vitro to a cell culture monolayer, the cells of the monolayer detach and clump together but remain viable. The detachment of the cell monolayers in vitro is thought to be analogous to the sloughing of vaginal epithelial cells seen in the vaginal mucosa during acute infections (98). CDF activity is probably a factor in pathogenesis, since *Pentatrichomonas hominis*, a nonpathogenic species, does not show CDF activity (98).

CDF, which is thought to be an extracellular factor (98), was found to be a 200-kDa glycoprotein (98) which is heat and acid labile (98, 240). The concentration of CDF in the filtrates varied with three factors: the duration of *T. vaginalis* growth prior to filtrate preparation, the initial inoculum size, and the pH of the filtrate prior to harvesting (98). Activity was also found to be affected by pH: Pindak et al. (240) found the optimum pH of the cell-free filtrate to be around 7.2. Garber et al. (98), however, found that purified CDF was active within pH 5.0 to 8.5, with the optimum activity at pH 6.5 and inactivity below pH 4.5. This is of clinical relevance since the normal pH of the vagina is 4.5 but is greater than 5.0 during trichomoniasis. The rise in vaginal pH during trichomoniasis may therefore be crucial in the pathogenesis of the disease.

CDF levels have been shown to correlate with the severity of the clinical symptoms of vaginitis. Increasing production of CDF was associated with increased severity of clinical disease (96). CDF is also immunogenic, and the detaching activity is inactivated by human sera reactive to *T. vaginalis* (98). Thus, local vaginal antibodies may decrease the effects of CDF (96). It is not certain whether the regulation of CDF production (i.e., its concentration), its activity, its immunogenicity, or a combination of the three plays a role in the severity of symptoms. Indeed, all of the pathogenic mechanisms (i.e., contact dependent, contact independent, and immune response) are probably important in the virulence of this disease.

CDF production is likely to be influenced by the concentration of estrogen in the vagina. In vitro, the production of CDF by *T. vaginalis* has been shown to decrease in the presence of β-estradiol. The maximal decrease was shown at a β-estradiol concentration of 10^{-7} to 10^{-8} M, which is of clinical relevance since human systemic levels of β-estradiol are in the range of 10^{-8} to 10^{-9} M (99). This finding may explain some of the etiology of the disease, i.e., the worsening of symptoms around the time of menses, when estrogen levels are lowest. As well, it may explain why the application of estradiol pellets intravaginally seems to ameliorate the symptoms without eradicating the infection (185).

Several investigators have been unable to demonstrate a cytopathic effect with cell-free filtrates of *T. vaginalis* culture (19, 63, 164, 167, 245). *T. vaginalis* is known to excrete lactic and acetic acids in cell culture, and so unless the pH of the cell culture is maintained, the pH drops below 4.5 (96, 98, 240). The intolerance of CDF to acidic pH may explain why these investigators could not demonstrate its activity (94, 98, 240).

Interaction with the Vaginal Flora

The establishment of *T. vaginalis* in the vagina is puzzling indeed, since the normal pH of the vagina is a very acidic 4.5 while this organism thrives in a less acidic pH of >5. The relationship between protective lactobacilli and *T. vaginalis* is not completely understood.

The rise in the pH of the vagina is also marked, with a concomitant reduction (or complete loss) of *Lactobacillus acidophilus* and an increase in the proportion of anaerobic bacteria. It appears that in vitro, with a controlled pH, lactobacilli do not affect the growth of *T. vaginalis*; however, the parasite seems to have a deleterious effect on *L. acidophilus* (207). Several mechanisms have been proposed: *T. vaginalis* has been observed to phagocytize bacteria (102, 274), and this may occur with lactobacilli as well. Another hypothesis is that products, such as CDF or proteinases secreted by *T. vaginalis*, may destroy the lactobacilli (206).

Immune System Evasion

In a hostile changing environment, *T. vaginalis* can survive and flourish. Its ability to evade the host immune system is an important aspect of pathogenesis. Avoidance of complement is a strategic tactic which is used by *T. vaginalis* to overcome the human immune system. It has long been known that *T. vaginalis* activates the alternative pathway of complement (105), yet we are only just beginning to understand how the parasite escapes eradication. *T. vaginalis* has taken advantage of a niche in which little complement is present. Cervical mucus is surprisingly deficient in complement (20, 78). Menstrual blood represents the only source of complement available to the vagina. Interestingly, its complement activity is about half that of venous blood, and about one-third of menstrual blood samples have no complement activity at all (20, 78). Menstrual blood has appreciable complement-mediated cytotoxicity toward *T. vaginalis*, and although a reduction in parasite concentration is seen during menses, trichomonal infection persists even after menses (77). While the number of organisms in the vagina actually decreases during menses, it is the virulence factors discussed above, many of which are mediated by iron, which contribute to the exacerbation of symptoms at this time.

To this end, it was found that iron was a contributing factor in complement resistance. Demes et al. (78) found that fresh isolates of *T. vaginalis* differ in their susceptibility or resistance to complement-mediated lysis in serum. It appears that complement-resistant fresh isolates become susceptible to complement after prolonged in vitro cultivation (78), which is consistent with the hypothesis that phenotypic variation allows the trichomonad to avoid lysis by complement (20). Resistance to complement is dependent upon a high concentration of iron (20), a nutrient which is indeed abundant during menses. It seems that iron upregulates the expression of CPs, which have been found to degrade the C3 portion of complement on the surface of the organism; this allows the organism to evade complement-mediated destruction (20).

Like many other protozoan parasites, *T. vaginalis* displays phenotypic variation as a mechanism of immune evasion. Alderete et al. (9) have found that two classes of markers are alternately expressed on the surface of the organism: the highly immunogenic glycoproteins (P270) and the adhesins (AP65, AP51, AP33, and AP23) (9). While all isolates (type I and type II) synthesize P270, only type II organisms can undergo phenotypic variation between cytoplasmic and cell surface expression of P270 (8). Thus, the phenotypes are described as $A^+ B^-$ (P270 positive) and $A^- B^+$ (P270 negative) (8). The positive-phenotype organisms lack adhesins and cannot cytoadhere or

parasitize host cells (8). Only the organisms of the negative phenotype, which express the adhesins, have the ability to cytoadhere (8, 16). It seems that in vivo, an antigenic shift occurs from the positive to the negative phenotype (6, 11, 16). After prolonged cultivation in vitro, the organisms shift toward the positive phenotype (8).

The P270 glycoprotein has been shown to have a single repetitive DREGRD epitope, which is important for antibody binding (71), and it was found that some organisms bearing the P270 antigen on the cell surface are susceptible to antibody-mediated, complement-independent lysis in vitro (15). The lack of P270 surface expression and the immunorecessive nature of the adhesins permit the negative-phenotype organisms to survive antibody attack (6). The adhesin proteins appear to mimic the structure of malic enzyme, which may account for their poor immunogenicity (18, 87). This molecular mimicry is yet another example of how the trichomonad can escape detection by the immune system (87).

Another high-molecular-weight immunogen, P230, is present on the surface of all parasites but undergoes conformational changes which allow it to evade antibody (11). This was termed epitope phenotypic variation. Vaginal IgG, which is reactive to P230 of *T. vaginalis*, was not cytolytic, even in the presence of complement (17). Furthermore, the antibody response was restricted to only one or a few epitopes on the 230-kDa protein (17), allowing the organism to evade opsonization.

In addition to these mechanisms, *T. vaginalis* has numerous other ways of evading the immune system. Provenzano and Alderete (243) have reported that numerous CPs secreted by *T. vaginalis* degrade IgG, IgM, and IgA, which allows the organism to survive the antibody response. This parasite also secretes copious amounts of highly immunogenic soluble antigens (12). A continuous release of these antigens may neutralize antibody or cytotoxic T lymphocytes, thus short-circuiting specific anti-*T. vaginalis* defense mechanisms (12). As well, *T. vaginalis* can coat itself with host plasma proteins. This coating does not allow the hosts immune system to recognize the parasite as foreign (236). Thus, immune system mechanisms such as antigen presentation and complement-mediated lysis will not occur.

T. vaginalis RNA Virus

Interestingly, only the P270-positive phenotype organisms have been found to harbor a double-stranded RNA virus (154, 297), termed *T. vaginalis* RNA virus (TVV) (298). It seems that P270 surface expression is correlated with the presence of TVV (154, 297), since the loss of P270 surface expression was associated with the loss of TVV (16, 154, 297). It has been suggested the presence of TVV may even upregulate P270 mRNA accumulation (154). This correlation, however, does not explain two independent paradoxical observations: during prolonged cultivation in vitro, a transition toward P270 surface expression is seen (8), yet TVV is associated only with fresh isolates and is lost after prolonged in vitro cultivation (154, 261, 297). Although it appears that TVV is not associated with complement-mediated lysis (261), the relationship between TVV, P270 surface expression, and antibody response independent of complement needs to be defined. Although preliminary characterization of the TVV genome has been reported (153, 155, 276), the role that TVV plays in the pathogenicity and immunogenicity of *T. vaginalis* is uncertain.

ADAPTATION IN A CHANGING ENVIRONMENT

Clearly, *T. vaginalis* has developed several mechanisms for surviving in the ever-changing vaginal microenvironment. During menstruation, the environment undergoes a dramatic change with the influx of erythrocytes, host macromolecules, and serum constituents, as well as large changes in pH. It is remarkable that not only does the parasite survive these dramatic environmental changes but also the infection persists. The menstrual flow provides nutrients to the parasite, as well as a supply of iron, which is a key factor in gene regulation of *T. vaginalis*. As we have seen, iron upregulates various adhesins, immunogens, and C3-degrading proteinases which are instrumental in the ability of the parasite to deal with stresses in its host environment.

T. vaginalis has also developed mechanisms to deal with oxidative stress. Upon exposure to hydrogen peroxide, the parasite upregulates the production of various heat shock proteins ranging in size from 35 to 165 kDa (43, 74, 75). These act as chaperones (251) and also aid in stabilizing the cell during rapid upshifts and downshifts in the rate of protein synthesis caused by stress.

The presence of P-glycoprotein is also suggested to be involved in the stress response. This molecule is usually associated with multidrug resistance, but this has not been seen in *T. vaginalis*. P-glycoprotein is thought to be a transporter molecule (147), but its specific function in the *T. vaginalis* stress response has not been defined.

CONCLUSIONS

Although *T. vaginalis* was first described by Donné in 1836, research on this organism did not begin until the 20th century. The research has been a progression of phases throughout the last 60 years and has gone from developing axenic culture and defining nutritional requirements to finding an effective treatment. In the 1960s and 1970s, research focused on biochemical tests and microscopic examination to understand the growth characteristics and behavior of the organism. It was not until the 1980s that immunologic methods and molecular biological techniques became available and were applied to study the pathogenesis and immunology of this organism. Research involving these techniques has provided much-needed information on identification and characterization of many virulence factors of *T. vaginalis*. However, the quest to understand its pathogenesis has only just begun.

Trichomoniasis is not merely a nuisance disease of women. It is an unpleasant, irritating, and potentially dangerous disease that can go undiagnosed for years and is often passed on by an asymptomatic carrier. It is the world's most common nonviral STD, and it is strongly associated with several complications in pregnancy and with an increase in the transmission of HIV.

Certainly, treatment of all infected individuals, whether symptomatic or asymptomatic, as well as public education and prevention programs will help curb the spread of the disease. However, metronidazole resistance of trichomonads is on the rise, and there may be a time when metronidazole is no longer effective. Furthermore, metronidazole cannot be used for all individuals. New treatments outside of the nitroimidazole family are clearly needed. However, an effective vaccine would be particularly welcome, especially in areas of high prevalence of disease. Only when the mechanisms of pathogenesis have been well defined will we be able to develop new strategies to control this disease.

T. vaginalis is a very complex organism, from its biochemistry

to the mechanisms of pathogenesis. Areas of pathogenesis that should be pursued include defining soluble factors, further elucidating the contact-dependent relationship between the vaginal epithelium and *T. vaginalis*, and defining how the organism can establish itself in a normally inhospitable and changing environment. It will also be important to further define the role of the human immune system in trichomoniasis in order to develop targeted intervention strategies. With continued collaboration and cooperation within the scientific community, we may one day understand the pathogenesis of *T. vaginalis* well enough to develop a safe, effective, and cost-effective vaccine.

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