Biology of trichomonosis

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Trichomonas vaginalis is emerging as a major pathogen of men and women and is associated with serious health consequences. Advances in diagnosis and treatment are presented. The complexity of trichomonad pathogenesis is illustrated in the interaction of this parasite with human cells, tissues and the immune system. It is now becoming evident that the interaction of trichomonads with the host is frequently modulated by environmental signals. The molecular biology of trichomonads is still in its infancy, but analysis of genes, genomic structure and transcriptional mechanisms suggest that trichomonads combine both prokaryotic and eukaryotic features. Evidence for the ancient divergence of trichomonads from other eukaryotic lineages is discussed. Curr Opin Infect Dis 13:37–45. © 2000 Lippincott Williams & Wilkins.

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Abbreviations

SLPI	secretory leukocyte protease inhibitor
INR	metazoan initiator element

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Introduction

Trichomonas vaginalis, despite its status as a stepchild of sexually transmitted infections, has numerically never been a minor sexually transmitted infection [1•]. Primarily considered to be a cause of vaginitis in women, trichomonads have been frequently dismissed as a nuisance parasite. Data, although slowly forthcoming, however, paint a very different picture. Recent publications indicate that this parasite's impact is not only limited to vaginitis but is also a major factor in promoting transmission of HIV [2•,3•], in causing low-weight and premature birth [4], and in predisposing women to atypical pelvic inflammatory disease [5], cervical cancer [6–8] and infertility [9]. This parasite may also contribute to long-lasting morbidity of women and children born to infected mothers [1•]. Finally, the recent finding of high rates of trichomonosis among men highlights the tremendous impact this parasite has on all sexually active persons, regardless of gender [10••]. Clearly, these new trends warrant a new interest and renewed efforts in understanding the biology of pathogenic trichomonads. The emerging themes of trichomonosis research over the past 2 years are thus the focus of this review. For a more comprehensive overview of the field, the reader is directed to the excellent recent reviews by Petrin *et al.* [11••] and Alderete [12••].

Clinical aspects of trichomonosis

Trichomonosis is now the most common non-viral sexually transmitted disease in the world [13••]. Trichomonal vaginitis has been reported in 50–75% of female sex workers, in up to 50% of female inmates, in 10–25% of women attending gynecological, sexually transmitted disease or family planning clinics and in 13% of pregnant women in the US [14–17]. Prevalence rates in sub-Saharan Africa have been reported to be as high as 46% in women of childbearing age [10••]. World wide, 107 million cases of trichomonosis are reported with the majority (92%) occurring in women [13••]. It should be noted that the incidence of trichomonosis in men is most likely grossly underestimated due the insensitivity of wet-mount preparations [18•,19]. Using a combination of wet-mount and polymerase chain reaction a recent study noted a prevalence of 20.8% and 12.2% in symptomatic and asymptomatic men, respectively [10••]. These surprising results highlight that trichomonosis can no longer be considered a cause of morbidity of women alone. It is estimated that annually 167 million people (84 million men and 83 million women) will become infected with *T. vaginalis*; of these 10–50% of women and 15–50% of men will be asymptomatic [13••].

Interestingly, the prevalence of trichomonosis in African American populations in the United States is significantly higher than that of any other ethnic or racial group [20]. No biological or behavioral reasons were known why ethnicity alone would alter the risk for acquisition of trichomonosis. A revolutionary study by Lauman and Yoosik-Youm may have solved this enigma [21••]. In their remarkable study, these authors found that the patterns of sexual networks can explain the increased risk for sexually transmitted infections among ethnic groups. In the case of African Americans, increased sexual contact between the core group and the periphery leads to the spread of infection into the population, whereas sexual segregation from other racial groups results in the maintenance of infection within this population [21••,22•].

Infection with *Trichomonas vaginalis* has major health consequences for women, including predisposition to HIV, association with cervical cancer and pelvic inflammatory disease, and complications of pregnancy [11••]. The vaginal epithelium is the primary site of infection. A hallmark and complication among patients with trichomonosis is the wide variations in symptomatology [15,23,24]. While some patients are characterized as asymptomatic, women with trichomonal vaginitis (70%) frequently experience vaginal discharge due to a leukocytic infiltration. The consistency of the discharge varies from patient to patient from thin and scanty to profuse and thick. The classical symptom of profuse frothy yellow discharge, however, occurs in only 10–30% of women. Similarly, the vagina and cervix of patients with trichomonosis may be erythematous and edematous, with general erosion of the cervical epithelium and punctate hemorrhages on the cervical wall, termed 'colpitis macularis' or 'strawberry cervix'. Although highly specific for trichomonosis, the strawberry cervix is nonetheless present in only a few (2–5%) women. Sharp abdominal pain has been documented among some patients with trichomonosis and may be indicative of infection in the upper urogenital region, possibly involving regional lymphadenopathy and salpingitis [25••].

In contrast to women, men become infected following recent contact with an infected partner but, for unknown reasons, may have a self-limiting infection [24]. Non-gonococcal non-chlamydial urethritis is the most common complaint of men infected with *T. vaginalis*. Incidence rates of trichomonal urethritis appear to be on the rise, especially in geographic areas where gonococcal infections are on a decline [1•]. The presence of trichomonads in the prostate gland in men with trichomonosis has been reported [26]. However, the role of infection in prostatitis and/or in infertility or numerous other reported sequelae is unclear.

Syndromic patient management algorithms of sexually transmitted disease syndromes have been used successfully for the diagnosis of many sexually transmitted infections but have performed poorly for *T. vaginalis* [27•]. Nevertheless diagnosis of trichomonosis is still often based on clinical presentation and especially the characteristics of the vaginal discharge [18•,28]. However, symptomatology and the presence of the classical signs of trichomonad infections are usually poor predictors of disease [18•]. A newly developed algorithm based on risk assessment, symptoms, speculum examination and microscopy with a sensitivity of 92.4% and a 100% positive predictive value, however, offers hope that syndromic management of *T. vaginalis* infections can be significantly improved [29].

Positive diagnosis of trichomonosis is generally established by wet-mount examination, but this method is only 30–80% sensitive compared to the gold standard of culture. Culturing is laborious, frequently inaccessible and often cost prohibitive for many clinical settings, and thus is not routinely used [5,30]. The InPouchTV culture system, equally sensitive as traditional culturing in many clinical settings, has been shown to be a cost-effective and sensitive alternative [31•]. Cost of diagnosis can be further reduced, by delaying inoculation into culture media, until wet-mount results become available. No significant difference in the sensitivity of the InPouchTV culture system between delayed and immediate inoculation of culture medium has been demonstrated [32•].

Other diagnostic techniques such as enzyme-linked immunosorbent assay [33], hybridization [34] and fluorescent antibody tests [19] have been used to detect this parasite and have reported sensitivities between 70% and 90%. The advent of polymerase chain reaction opened a new avenue for diagnosis. Several polymerase chain reaction diagnostic tests with sensitivities and specificities approaching 100% have been recently developed [18•,35]. Importantly, polymerase chain reaction diagnostics are easily adaptable to self-administered sampling, which in turn is useful due to the ease of specimen collection and patient compliance. In this regard, the tampon test has proved particularly useful. This test not only had high sensitivity and specificity, but was even useful on specimens where culture and wet mount failed to detect any trichomonads [36•,37•]. With further developments in polymerase chain reaction based diagnostics, speculum-free evaluation of vaginal infections should become a reality in the near future [38•].

Treatment options are still limited but exciting progress in the biochemical characterization of trichomonads hint at the development of new drugs, targeting novel and unique trichomonad structures [39,40•,41•,42••,43••,44,45,46•,47•,48,49,50•,51•]. Metronidazole (Flagyl) or derivatives thereof are the most commonly used drugs. Treatment consists of either a single 2 g dose of metronidazole or 400 mg of metronidazole twice daily for 5–7 days given orally [25••]. Vaginal application of metronidazole may provide symptomatic relief but will not cure the infection [52•]. In the past, metronidazole was contraindicated during pregnancy because of the concern about possible teratogenic effects [53–55]. The strong association of trichomonosis with premature delivery and low-birth weight, however, suggests the desirability of treatment. Indeed, the preponderance of evidence shows that the benefits of metronidazole treatment outweigh by far the risks of using this drug. First, teratogenic effects of metronidazole in animal species have not been demonstrated. Second, several studies have shown no or a very slight risk of metronidazole treatment during pregnancy [53–55]. Finally, a large study of 1706 pregnant women given metronidazole showed conclusively that oral metronidazole treatment during pregnancy is not significantly associated with congenital abnormalities [56••]. In spite of overwhelming medical evidence of the safety of metronidazole, treatment during the first trimester must be considered carefully because of possible legal liability.

Treatment failure is not uncommon and may be the result of non-compliance, reinfection or colonization with a metronidazole-resistant isolate of trichomonad. It appears that, at least since 1995, the majority of clinically resistant trichomonosis results from metronidazole resistant isolates [57]. Furthermore, the incidence of metronidazole-resistant trichomonosis may also be increasing [11••]. Treatment of such cases is often problematic. In many cases increased dosages of metronidazole may resolve the infection. Otherwise, a variety of treatment regimes have been utilized that may or may not work [58•,59]. A clear systematic and scientific examination of treatment alternatives does not exist.

It is clear that there is a need for a safe, systemic and non-imidazole based drug alternative for pregnant women, people with metronidazole-resistant trichomonosis, or for patients having allergic reactions to this group of drugs. Several promising leads toward this goal have recently been reported. Firstly, several new 5-nitroimidazole and 1-lactam-substituted nitroimidazole compounds have been shown to be highly effective against metronidazole-resistant trichomonads [60•]. Secondly, disulfiram and its first mammalian metabolite ditiocarb, both used in alcoholism treatment, have also been shown effective against metronidazole resistant trichomonads *in vitro* [61•]. Finally, the findings of alternative trichomonad keto-acid oxidoreductases [41•], expression of chitin at the trichomonad cell surface [62•] and expression of a thymidine kinase [50•] may lead to the design of new antitrichomonal drugs. Particularly intriguing and promising is the ability of deoxyuridine analogs to inhibit *T. vaginalis* thymidine kinase, and consequently prevent proliferation of this parasite [50•].

Trichomonas vaginalis and HIV

The correlation between trichomonosis and increased risk of acquiring HIV (up to 5-fold) has been firmly established [63–68]. Even at such a modest increased risk, HIV community transmission can be increased significantly because of the high prevalence of trichomonosis within the general population, and in particular within risk groups [2•,69•]. Presently, the increased risk appears to be present for the receptive partner only. The mechanisms of acquisition enhancement are unknown, but suggestions have included erosion of the mucosa by the parasite and/or the inflammatory response, and recruitment of HIV target cells into the genital area [70•]. The complexity of these associations, however, is now becoming clear. Not only do trichomonads disrupt the epithelial barrier but also appear to recruit CD4 T-lymphocytes to the genital area [71•]. Increased secretion of cytokines (interleukins 1, 6, 8 and 10), known to increase HIV susceptibility, has now been demonstrated during trichomonosis [72,73]. Furthermore, the degradation of secretory leukocyte protease inhibitor, known to prevent HIV transmission through the oral mucosa, by trichomonad proteinase is yet another mechanism to enhance HIV transmission [42••]. Another important and unexpected factor in the risk for HIV acquisition is the demonstration of significantly increased viral loads in semen of men with trichomonal urethritis [10••]. Clearly, these new findings strongly suggest that aggressive screening and treatment for trichomonal infection of men and women in risk groups may significantly reduce community transmission of HIV.

Interactions between trichomonads and host cells

Receptor–ligand-type interactions are involved between trichomonads and host epithelial cells. The surface structures on vaginal epithelial cells recognized by *T. vaginalis* organisms are unknown. Four trichomonad surface proteins have been identified as mediating cytoadherence. The synthesis of the four adhesins was coordinately up-regulated by binding to epithelial cells and by iron [74–76,77•].

More recent work reveals that three of the four adhesins studied to date are each members of multi-gene families [78•]. Sequence analyses at both the nucleotide and amino acid levels revealed structural similarity of adhesins with known metabolic enzymes [79,80]. Analysis of the receptor-binding epitope for the adhesin AP33 identified a 24-amino acid binding domain with the ability to inhibit parasite associations with host cells [43••].

The involvement of carbohydrate structures in cytoadherence has also been suggested, but remains problematic. A quantitative electron microscopy study showing the involvement of trichomonad surface-associated glycoconjugates in cytoadherence raises the possibility of the presence of lectin-like adhesins in *T. vaginalis* [81•]. A similar study, however, demonstrated that cell surface glycoconjugates are not involved in cytoadherence [82•]. The use of different target cells may be an explanation for these contradictory results. It should be noted that the protein adhesins are functional when tested with in-vivo relevant target cells.

Initial binding to host cells is followed by transformation of the parasite from its ellipsoid to amoeboid form [83]. The ability to undergo such a dramatic morphological transformation clearly requires a rearrangement of the cytoskeleton. Alpha-actinin, an actin binding protein that participates in rearrangements of actin fibers, has now been described in trichomonads. Its role in transformation is suggested by the finding that alpha-actinin, usually distributed throughout the cytoplasm, becomes localized to the periphery upon the change to the amoeboid form [84•].

Amoeboid transformation results in intimate contact of the parasite with the host cell. Extensive interdigitation and microchannel formation has been observed by electron microscopy [83]. Using a variety of electron microscopic techniques Furtado and Benchimol [85•] have extended these observations to include extensive plasma membrane fusion between *T. vaginalis* and vaginal epithelial cells. Although the roles of contact-dependent cytotoxins [86–89], proteinases [90] and phospholipase [91] have been well established in the lysis of host cells, membrane fusion may yet represent another mechanism leading to host cell damage.

It is reasonable to expect that cytoadherence to vaginal epithelial cells is not the only mechanism by which trichomonads adhere to host cell surfaces. For example, erosion of the vaginal epithelium as seen for colpitis macularis may allow access of parasites to the basement membrane and accompanying complex structures. Interestingly, the reports on the specific binding by *T. vaginalis* organisms to fibronectin [92] and laminin [92,93] may reflect associations with basement membrane sites. Importantly, the interaction of trichomonads with these extracellular matrix proteins appears to be mediated through the same binding domains as those utilized by the host. In the case of laminin, the amino acid sequence YIGSR is recognized by *T. vaginalis*, as it is by host cells [94•].

Finally, colonization of the vaginal epithelium requires trichomonads to breach the mucous covering. The interaction of trichomonads with mucus and subsequent penetration and parasitism of the underlying vaginal cells have been shown to be very complex. Mucin adhesins, proteinase activity and flagellar movement all appear to be necessary to traverse the mucous layer [95•].

Evasion of host immune responses

Cellular and humoral immune responses are evident in patients with trichomonosis, but are not protective [11••]. Although not found in all patients with trichomonosis, increased numbers of polymorphonuclear leukocytes can be detected readily in secretions [96]. Interestingly, while both leukocytes and macrophages in addition to antibody [97] and complement [98,99] can eliminate the parasites, it is clear that *T. vaginalis* has effectively neutralized the host immune surveillance system. Further, hydrogen peroxide-producing lactobacilli are considered protective normal vaginal flora [100•,101].

The numerous cysteine proteinases synthesized by *T. vaginalis* contribute significantly to immune evasion. The cysteine proteinases are cytotoxic [90] and hemolytic [88]. All subclasses of immunoglobulins are susceptible to the trichomonad cysteine proteinases [48,102,103]. Similarly, parasites are resistant to complement-mediated lysis by the action of at least one cysteine proteinase induced by high iron growth conditions. The proteinase degrades C3 deposited on *T. vaginalis* surfaces [98]. Secretory leukocyte protease inhibitor (SLPI) is yet another factor protecting mucosal surfaces. Again, trichomonad proteinases are able to degrade SLPI and render it non-functional. Interestingly, SLPI also has been shown to prevent HIV transmission, thus trichomonad proteinases may be partly responsible for the observed increase in risk of HIV acquisition in women with trichomonosis [42••]. Lastly, hydrogen peroxide readily neutralizes the cysteine proteinases, showing the protective effect of lactobacilli normal flora. However, displacement of the lactobacilli immediately following infection with *T. vaginalis*, through phagocytosis, may subvert this host protective effect [100•].

Leukocytic infiltration is frequently seen in women with trichomonosis. Although, leukocytes readily kill trichomonads *in vitro*, at parasite to leukocyte ratios of greater than 1:3000, they do not appear to be protective *in vivo*. However, at low parasite to leukocyte ratios, initial contact between parasite and leukocyte results in phagocytic pseudopod formation, internalization, and finally degradation of the immune cell in parasite phagocytic vacuoles [100•].

Phenotypic variation, another immune evasion mechanism for *T. vaginalis*, is defined on the basis of surface versus cytoplasmic expression of a repertoire of high M_r immunogens. A representative of these immunogens termed P270 has been extensively studied. From fluorescence experiments it has been observed that two types of isolates occur naturally during infections with *T. vaginalis*. Type I isolates were comprised of homogeneous populations of non-fluorescent trichomonads that synthesize and express P270 only in the cytoplasm. In contrast, type II isolates were heterogeneous with subpopulations of both fluorescent and non-fluorescent parasites and were able to switch between the fluorescent and non-fluorescent state [104–106].

The *p270* gene for one *T. vaginalis* isolate T068-II has now been sequenced [107•]. Consistent with earlier reports a significant portion of the *p270* gene has a 333-bp unit tandemly repeated 18 times that contains the epitope DREGRD detected by patient antibody. The non-repeat coding regions for the 5′- and 3′-ends are 69 nucleotides (23 amino acids) and 1183 nucleotides (395 amino acids), respectively. The start codon is immediately preceded by a 12-nucleotide sequence that has significant homology to the recently described Inr sequence of trichomonad promoters. A leader sequence and a transmembrane domain are also present. This gene appears to be highly conserved among trichomonads but the number of repeat units may vary [107•]. This single-copy gene was furthermore shown to be up-regulated by low iron conditions and highly phosphorylated under high iron conditions [108•].

Environmental adaptations

The vagina is one of the most complex sites of infection for a mucosal pathogen. This host environment is constantly changing under the influence of the menstrual cycle. The vagina is a nutrient-limiting site that cannot promote the 4–6-hour generation time seen during in-vitro growth of habituated parasites in a serum-based, trypticase and yeast extract complex medium [109]. It is thus not surprising that biological and biochemical properties of these in-vitro grown parasites may be different from those at the site of infection. Indeed, the availability of iron in growth media already has been shown to significantly affect the physiology of trichomonads [76,109,110]. Even a simple change in commonly accepted growth media appears to have a physiological consequence [111]. Further underscoring the tremendous adaptability of this parasite to challenging environmental pressures is the demonstration that trichomonads respond to changes in growth rate and carbon source with a change in mRNA levels and activity of glycolytic enzymes [112•]. In light of these findings, it may not be surprising that inconsistent results, depending on the growth condition under otherwise comparable experimental set-ups, may be obtained. Even more important, these data also suggest that trichomonads possess signal transduction pathways that link changes in the environment with appropriate changes in transcriptional and post-transcriptional regulatory mechanisms. The exploration of such regulatory networks will be crucial in understanding this parasite–host interaction.

Molecular biology of trichomonads

Progress in our understanding of nucleic acid driven processes in *T. vaginalis* is excruciatingly slow. Despite this fact, several themes in this area of research seem to be emerging. First, all genes cloned from trichomonads are monocistronic. Second, genes are frequently present as multicopy genes, such as the trichomonad adhesins and the pyrophosphate-dependent phosphofructokinase. Third, homologues of genes have arisen either due to gene duplication, or are of polyphyletic origin. For example, the eukaryotic family of B DNA polymerases is suggested to have arisen by gene duplication; in contrast, the two forms of HSp70 may have arisen from two diverse origins. Finally, trichomonad genes appear to be devoid of introns. However, the discovery of a functional spliceosomal protein, PRP8, may indicate that at least some genes contain introns or that trichomonads may perform limited trans-splicing [113••].

New developments in this area have focused on transcription initiation and genomic structure. Despite considerable efforts, typical eukaryotic core promoter elements have not been found in trichomonads. An analysis of 33 genes has revealed that a consensus sequence resembling a metazoan initiator element (Inr) is located within 6–20 nucleotides upstream from the translational initiation codon. Transcriptional fusions and primer extension experiments established that the Inr invariably determines the start site of transcription. Interestingly, and in contrast to other metazoans, the trichomonad Inr is located very close to the translational start codon, resulting in unusually short 5′-untranslated regions for trichomonad mRNAs [114••].

The genomic structure of trichomonads has been examined by pulsed field gel electrophoresis. Six chromosomal elements that fall into three size classes have been identified. The size and number of chromosomes of 15 *T. vaginalis* isolates were found to be identical, suggesting that genetic heterogeneity among trichomonads does not involve major chromosomal rearrangements [115•].

Molecular phylogeny

The amitochondriate protist *Trichomonas vaginalis* has been suspected to be a member of the earliest branching eukaryotes. The finding of unusual trichomonal structures and biochemical pathways such as the unique posttranslational glutamylation of tubulins [116], the unique sulfur amino acid metabolism [46•], the spectroscopically atypical hydrogenosomal ferredoxin [45], the presence of novel iron superoxide dismutase [51•], and the centriole-like behavior of trichomonad flagella through the progression of the cell cycle [117•], all certainly confirm this assertion.

Phylogenies based on the sequence of the small rRNA subunit show that trichomonads emerge at the base of the eukaryotic tree [118,119,120•]. In contrast, phylogenies based on protein sequences often suggest a later divergence. Recently, phylogenetic analysis of DNA sequences of glyceraldehyde-3-phosphate dehydrogenase [121•], alpha-1 elongation factor [122•], DNA polymerase [123•], pyrophosphate-dependent phosphofructokinase [47•], heat-shock proteins [120•,124,125] and valyl-tRNA synthetase [126•] all support an early branching point for trichomonads. However, the presence of mitochondrial-like heat shock proteins (HSP60 and HSP70), in addition to the presence of nuclear-coded valyl-tRNA synthetase [126•], indicate that the branch point occurred some point after acquisition of the mitochondrial endosymbiont.

Unfortunately, most studies also show a lack of resolution in interphyla relationships among the early branching parabasalids, diplomonads and microsporidia. The reason for the inability to resolve clearly the evolutionary relationships among these organisms is unclear but may be due to recombination, lateral gene transfer, inversions, fusions, chimeric origins, gene duplication and convergent evolution. Data on alpha-1 elongation factor and HSP70, in addition, are also consistent with the notion of a simultaneous diversification of major eukaryotic lineages [120•,122•]. Importantly, taken together, these data strongly imply that the biochemistry of trichomonads is unique and sufficiently different from higher eukaryotes to provide ample targets for chemotherapeutic intervention.

Conclusion

Trichomonas vaginalis is a major pathogen of men and women, with tremendous health consequences for the infected individuals and their communities. Despite progress in our understanding of the evolutionary history, biochemistry, biology and pathogenesis of trichomonads that have led to the identification of many promising targets for drug development, treatment options are still very limited. A major reason for this is that preclinical and clinical studies are lagging behind these new and encouraging studies. A general lack of appreciation for the seriousness of this disease, once classified as a minor sexually transmitted infection, probably is yet another factor. This is particularly evident in the disturbing fact that the antiquated wet-mount for diagnosis of trichomonosis is still widely used, despite the known and documented insensitivity of this test. It is obvious that an education program for medical professionals and the population as a whole is needed urgently.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
- • of special interest
- •• of outstanding interest

1 Hook EW. *Trichomonas vaginalis* – no longer a minor STD. Sex Transm Dis
• 1999; 26:388–389.
Summary of the importance of trichomonosis in men in reference to Hobbs *et al.* [10••].

2 Sorvillo F, Kerndt P. *Trichomonas vaginalis* and amplification of HIV-1
• transmission. Lancet 1998; 351:213–214.
Letter stressing the importance of trichomonosis in the HIV epidemic.

3 Fleming DT, Wasserheit JN. From epidemiological synergy to public health
• policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. Sex Transm Inf 1999; 75:3–17.
A review of the scientific data on the role of sexually transmitted infections in transmission of HIV infection. Specific policy recommendations are made.

4 Cotch MF, Pastorek JG, Nugent RP, Hillier SL, Gibbs RS, Martin DH, *et al.* *Trichomonas vaginalis* associated with low birth weight and preterm delivery. The Vaginal Infections and Prematurity Study Group. Sex Transm Dis 1997; 24:353–360.

5 Heine P, McGregor JA. *Trichomonas vaginalis*: a reemerging pathogen. Clin Obstet Gynecol 1993; 36:137–144.

6 Gram IT, Macaluso M, Churchill J, Stalsberg H. *Trichomonas vaginalis* (TV) and human papillomavirus (HPV) infection and the incidence of cervical intraepithelial neoplasia (CIN) grade III. Cancer Causes Control 1992; 3:231–236.

7 Kharsany AB, Hoosen AA, Moodley J, Bagaratee J, Gouws E. The association between sexually transmitted pathogens and cervical intra-epithelial neoplasia in a developing community. Genitourin Med 1993; 69:357–360.

8 Zhang ZF, Begg CB. Is *Trichomonas vaginalis* a cause of cervical neoplasia? Results from a combined analysis of 24 studies. Int J Epidemiol 1994; 23:682–690.

9 Grodstein F, Goldman MB, Cramer DW. Relation of tubal infertility to history of sexually transmitted diseases. Am J Epidemiol 1993; 137:577–584.

10 Hobbs MM, Kazembe P, Reed AW, Miller WC, Nkata E, Zimba D, *et al.*
•• *Trichomonas vaginalis* as a cause of urethritis in Malawian men. Sex Transm Dis 1999; 26:381–387.
Demonstrates for the first time the tremendous prevalence of trichomonal urethritis in men using polymerase chain reaction. The increased HIV viral load in semen of men with trichomonosis is also demonstrated.

11 Petrin D, Delgaty K, Bhatt R, Garber G. Clinical and microbiological aspects of
•• *Trichomonas vaginalis*. Clin Microbiol Rev 1998; 11:300–317.
Excellent review of the field of trichomonosis.

12 Alderete JF. *Trichomonas vaginalis*: a model mucosal parasite. Rev Med
•• Microbiol 1999; 10:165–173.
Excellent review of the pathogenesis of *T. vaginalis*.

13 Gerbase AC, Rowley JT, Heyman DHL, Berkley SFB, Piot P. Global prevalence
•• and incidence estimates of selected curable STDs. Sex Transm Inf 1998; 74:S12–S16.
Estimates of the global prevalence and incidence of trichomonosis and other curable sexually transmitted diseases are given.

14 Klausner JD, Baer JT, Contento KM, Bolan G. Investigation of a suspected outbreak of vaginal trichomoniasis among female inmates. Sex Transm Dis 1999; 26:335–338.

15 Wolner-Hanssen P, Krieger JN, Stevens CE, Kiviat NB, Koutsky L, Critchlow C, *et al.* Clinical manifestations of vaginal trichomoniasis. JAMA 1989; 261:571–576.

16 Rein MF, Chapel TA. Trichomoniasis, candidiasis, and the minor venereal diseases. Clin Obstet Gynecol 1975; 18:73–88.

17 Shuter J, Bell D, Graham D, Holbrook KA, Bellin EY. Rates of and risk factors for trichomoniasis among pregnant inmates in New York City. Sex Transm Dis 1998; 25:303–307.

18 Madico G, Quinn TC, Rompalo A, McKee KT Jr, Gaydos CA. Diagnosis of
• *Trichomonas vaginalis* infection by PCR using vaginal swab samples. J Clin Microbiol 1998; 36:3205–3210.
Demonstrates the validity and necessity of using a more sensitive test for the diagnosis of trichomoniasis.

19 Krieger JN, Tam MR, Stevens CE, Nielsen IO, Hale J, Kiviat NB, Holmes KK. Diagnosis of trichomoniasis. Comparison of conventional wet-mount examination with cytologic studies, cultures, and monoclonal antibody staining of direct specimens. JAMA 1988; 259:1223–1227.

20 Cotch MF, Pastorek JG, Nugent RP, Yerg DE, Martin DH, Eschenbach DA. Demographic and behavioral predictors of *Trichomonas vaginalis* infection among pregnant women. The Vaginal Infections and Prematurity Study Group. Obstet Gynecol 1991; 78:1087–1092.

21 Lauman EO, Yoosik-Youm MA. Racial/ethnic group differences in the
•• prevalence of sexually transmitted diseases in the United States: A network explanation. Sex Transm Dis 1999; 26:250–261.
A revolutionary study that shows that social networks are as important as behavioral factors in the outcome of sexually transmitted infections.

22 Sevgi AO. Sexual network patterns as determinants of STD rates: paradigm
• shift in the behavioral epidemiology. Sex Transm Dis 1999; 26:262–264.
Excellent and thought provoking comments on Lauman's study.

23 Fouts AC, Kraus SJ. *Trichomonas vaginalis*: reevaluation of its clinical presentation and laboratory diagnosis. J Infect Dis 1980; 141:137–143.

24 Krieger JN, Jenny C, Verdon M, Siegel N, Springwater R, Critchlow CW, Holmes KK. Clinical manifestations of trichomoniasis in men. Ann Intern Med 1993; 118:844–849.

25 Sherrard J. National guidelines for the management of *Trichomonas vaginalis*.
•• Sex Transm Inf 1999; 75:S21–S23.
Excellent and concise summary of the most important features of trichomonosis.

26 Gardner WA, Culberson DE, Bennett BD. *Trichomonas vaginalis* in the prostate gland. Arch Pathol Lab Med 1986; 110:430–432.

27 Ryan CA, Courtois BN, Hawes SE, Stevens CE, Eschenbach DA, Holmes KK.
• Risk assessment, symptoms, and signs as predictors of vulvovaginal and cervical infections in an urban US STD clinic: implications for use of STD algorithms. Sex Transm Inf 1998; 74:S59–S76.
A comprehensive analysis of syndromic alogorithms for the management of vaginitis.

28 Sobel JD. Vaginitis. N Engl J Med 1996; 337:1896–1903.

29 Ryan CA, Courtois BN, Hawes SE, Stevens CE, Eschenbach DA, Holmes KK. Risk assessment, symptoms, and signs as predictors of vulvovaginal and cervical infections in an urban US STD clinic: implications for use of STD algorithms. Sex Transm Infect 1998; 74:S59–S76.

30 Heine RP, Wiesenfeld HC, Sweet RL, Witkin SS. Polymerase chain reaction analysis of distal vaginal specimens: a less invasive strategy for detection of *Trichomonas vaginalis*. Clin Infect Dis 1997; 24:985–987.

31 Ohlemeyer CL, Hornberger LL, Lynch DA, Swierkosz EM. Diagnosis of
• *Trichomonas vaginalis* in adolescent females: InPouch TV culture versus wet-mount microscopy. J Adolesc Health 1998; 22:205–208.
Demonstrates the ease of use of this culturing system and its superior sensitivity when compared to wet-mount preparation.

32 Schwebke JR, Venglarik MF, Morgan SC. Delayed versus immediate bedside
• inoculation of culture media for diagnosis of vaginal trichomonosis. J Clin Microbiol 1999; 37:2369–2370.
If cost of culturing trichomonads is an issue, this study shows that cost can be reduced by using wet-mount first and inoculate culture media with wet-mount negative specimens only.

33 Sharma P, Malla N, Gupta I, Ganguly NK, Mahajan RC. A comparison of wet mount, culture and enzyme linked immunosorbent assay for the diagnosis of trichomoniasis in women. Trop Geogr Med 1991; 43:257–260.

34 DeMeo LR, Draper DL, McGregor JA, Moore DF, Peter CR, Kapernick PS, McCormack WM. Evaluation of a deoxyribonucleic acid probe for the detection of *Trichomonas vaginalis* in vaginal secretions. Am J Obstet Gynecol 1996; 174:1339–1342.

35 Shaio MF, Lin PR, Liu JY. Colorimetric one-tube nested PCR for detection of *Trichomonas vaginalis* in vaginal discharge. J Clin Microbiol 1997; 35:132–138.

36 Paterson BA, Tabrizi SN, Garland SM, Fairley CK, Bowden FJ. The tampon test
• for trichomoniasis: a comparison between conventional methods and a polymerase chain reaction for *Trichomonas vaginalis* in women. Sex Transm Infect 1998; 74:136–139.
A reliable polymerase chain reaction test for trichomonosis, especially useful for areas where a significant delay between sample taking and analysis is unavoidable.

37 Tabrizi SN, Paterson BA, Fairley CK, Bowden FJ, Garland SM. Comparison of
• tampon and urine as self-administered methods of specimen collection in the detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis* in women. Int J STD AIDS 1998; 9:347–349.
This study shows that patients compliance can be improved by self-administered specimen collection.

38 Blake DR, Duggan A, Quinn T, Zenilman J, Joffe A. Evaluation of vaginal
• infections in adolescent women: can it be done without a speculum? Pediatrics 1998; 102:939–944.
This study stresses the importance for developing diagnostic tests especially for adolescents.

39 Addis MF, *et al.* Identification of *Trichomonas vaginalis* alpha-actinin as the most common immunogen recognized by sera of women exposed to the parasite. J Infect Dis 1999; 180:1727–1730.

40 Bricheux G, Coffe G, Pradel N, Brugerolle G. Evidence for an uncommon alpha-
• actinin protein in *Trichomonas vaginalis*. Mol Biochem Parasitol 1998; 95:241–249.
Another study demonstrating the unusual biochemistry of trichomonads.

41 Brown DM, Upcroft JA, Dodd HN, Chen N, Upcroft P. Alternative 2-keto acid
• oxidoreductase activities in *Trichomonas vaginalis*. Mol Biochem Parasitol 1999; 98:203–214.
Alternative keto acid reductase activity present in trichomonads may provide alternative energy production pathways that circumvent metronidazole activation.

42 Draper D, Donohoe W, Mortimer L, Heine RP. Cysteine proteases of
•• *Trichomonas vaginalis* degrade secretory leukocyte protease inhibitor. J Infect Dis 1998; 178:815–819.
Proteinases appear to be important virulence factors. This study further underscores this contention, by showing that trichomonad proteinase degrades host factors that may be important in host protection from infectious diseases such as HIV.

43 Engbring JA, Alderete JF. Characterization of *Trichomonas vaginalis* AP33
•• adhesin and cell surface interactive domains. Microbiology 1998; 144:3011–3018.
This study establishes the molecular basis of cytoadherence.

44 Han Q, Lenz M, Tan Y, Xu M, Sun X, Tan X, *et al.* High expression, purification, and properties of recombinant homocysteine alpha, gamma-lyase. Protein Expr Purif 1998; 14:267–274.

45 Liu HY, Germanas JP. NMR spectroscopic studies of the hydrogenosomal [2Fe–2S] ferredoxin from *Trichomonas vaginalis*: hyperfine-shifted 1H resonances. J Inorg Biochem 1998; 72:127–131.

46 McKie AE, Edlind T, Walker J, Mottram JC, Coombs GH. The primitive
• protozoon *Trichomonas vaginalis* contains two methionine gamma-lyase genes that encode members of the gamma-family of pyridoxal 5′-phosphate-dependent enzymes. J Biol Chem 1998; 273:5549–5556.
The structure and organization of methionine lyases in trichomonads is presented and the importance of a critical cysteine residue was established.

47 Mertens E, Ladror US, Lee JA, Miretsky A, Morris A, Rozario C, *et al.* The
• pyrophosphate-dependent phosphofructokinase of the protist, *Trichomonas vaginalis*, and the evolutionary relationships of protist phosphofructokinases. J Mol Evol 1998; 47:739–750.
The phosphofructokinase enzyme of trichomonads is most similar to the mitochondriate *Naegleria fowleri*, supporting again the notion that trichomonads secondarily lost their mitochondria.

48 Min DY, Hyun KH, Ryu JS, Ahn MH, Cho MH. Degradations of human immunoglobulins and hemoglobin by a 60 kDa cysteine proteinase of *Trichomonas vaginalis*. Korean J Parasitol 1998; 36:261–268.

49 Sanon A, Bories C, Loiseau PM. Chitinolytic activities in *Trichomonas vaginalis*. Parasite 1998; 5:75–78.

50 Strosselli S, Spadari S, Walker RT, Basnak I, Focher F. *Trichomonas vaginalis*
• thymidine kinase: purification, characterization and search for inhibitors. Biochem J 1998; 334:15–22.
A study demonstrating the potential of thymidine kinase as a new target for the design of antitrichomonal drugs.

51 Viscogliosi E, Delgado-Viscogliosi P, Gerbod D, Dauchez M, Gratepanche S,
• Alix AJ, Dive D. Cloning and expression of an iron-containing superoxide dismutase in the parasitic protist, *Trichomonas vaginalis*. FEMS Microbiol Lett 1998; 161:115–123.
This study establishes the presence of a multigene family of iron-containing superoxide dismutases, which may have arisen by an endosymbiotic event.

52 duBouchet L, McGregor JA, Ismail M, McCormack WM. A pilot study of
• metronidazole vaginal gel versus oral metronidazole for the treatment of *Trichomonas vaginalis* vaginitis. Sex Transm Dis 1998; 25:176–179.
This study shows that metronidazole gel is useful to alleviate symptoms but not for curing the infection.

53 Roe FJ. Toxicologic evaluation of metronidazole with particular reference to carcinogenic, mutagenic, and teratogenic potential. Surgery 1983; 93:158–164.

54 Murphy PA, Jones E. Use of oral metronidazole in pregnancy. Risks, benefits, and practice guidelines. J Nurse Midwifery 1994; 39:214–220.

55 Burtin P, Taddio A, Ariburnu O, Einarson TR, Koren G. Safety of metronidazole in pregnancy: a meta-analysis. Am J Obstet Gynecol 1995; 172:525–529.

56 Czeizel AE, Rockenbauer M. A population based case-control teratologic study
•• of oral metronidazole treatment during pregnancy. Br J Obstet Gynaecol 1998; 105:322–327.
An important study demonstrating that the risk of metronidazole treatment during pregnancy is insignificant. The use of metronidazole treatment during pregnancy is warranted because of the risk of negative pregnancy outcome due to trichomonosis.

57 Sobel JD, Nagappan V, Nyirjesy P. Metronidazole-resistant vaginal trichomoniasis – an emerging problem. N Engl J Med 1999; 341:292–293.

58 Pattman RS. Recalcitrant vaginal trichomoniasis. Sex Transm Infect 1999;
• 75:127–128.
Treatment options for metronidazole resistant cases are given.

59 Lossick JG, Muller M, Gorrell TE. In vitro drug susceptibility and doses of metronidazole required for cure in cases of refractory vaginal trichomoniasis. J Infect Dis 1986; 153:948–955.

60 Upcroft JA, Campbell RW, Benakli K, Upcroft P, Vanelle P. Efficacy of new 5-
• nitroimidazoles against metronidazole-susceptible and -resistant *Giardia*, *Trichomonas*, and *Entamoeba* spp. Antimicrob Agents Chemother 1999; 43:73–76.
The effectiveness of a new class of imidazoles effective against metronidazole resistant trichomonads are discussed.

61 Bouma MJ, Snowdon D, Fairlamb AH, Ackers JP. Activity of disulfiram
• (bis(diethylthiocarbamoyl)disulphide) and ditiocarb (diethyldithiocarbamate) against metronidazole-sensitive and -resistant *Trichomonas vaginalis* and *Tritrichomonas foetus*. J Antimicrob Chemother 1998; 42:817–820.
A report showing the usefulness of another class of antitrichomonals.

62 Kneipp LF, *et al.* *Trichomonas vaginalis* and *Tritrichomonas foetus*: expression
• of chitin at the cell surface. Exp Parasitol 1998; 89:195–204.
This study shows that chitin is a structural component of the trichomonad surface.

63 Augenbraun MH, McCormack WM. Sexually transmitted diseases in HIV-infected persons. Infect Dis Clin North Am 1994; 8:439–448.

64 Estambale BB, Knight R. Protozoan infections and HIV-1 infection: a review. East Afr Med J 1992; 69:373–377.

65 Laga M, Manoka A, Kivuvu M, Malele B, Tuliza M, Nzila N, *et al.* Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: results from a cohort study. AIDS 1993; 7:95–102.

66 Le Bacq F, Mason PR, Gwanzura L, Robertson VJ, Latif AS. HIV and other sexually transmitted diseases at a rural hospital in Zimbabwe. Genitourin Med 1993; 69:352–356.

67 ter Meulen J, Mgaya HN, Chang-Claude J, Luande J, Mtiro H, Mhina M, *et al.* Risk factors for HIV infection in gynaecological inpatients in Dar es Salaam, Tanzania, 1988–1990. East Afr Med J 1992; 69:688–692.

68 Wilson TE, Jaccard J, Levinson RA, Minkoff H, Endias R. Testing for HIV and other sexually transmitted diseases: implications for risk behavior in women. Health Psychol 1996; 15:252–260.

69 Sorvillo F, Kovacs A, Kerndt P, Stek A, Muderspach L, Sanchez-Keeland L.
• Risk factors for trichomoniasis among women with human immunodeficiency virus (HIV) infection at a public clinic in Los Angeles County, California: implications for HIV prevention. Am J Trop Med Hyg 1998; 58:495–500.
This study suggests that HIV infected women are at increased risk for acquisition of trichomonosis.

70 Sutton MY, Sternberg M, Nsuami M, Behets F, Nelson AM, St Louis ME.
• Trichomoniasis in pregnant human immunodeficiency virus-infected and human immunodeficiency virus-uninfected Congolese women: prevalence, risk factors, and association with low birth weight. Am J Obstet Gynecol 1999; 181:656–662.
The findings of this study show that low birth weight in women with trichomonosis is independent of HIV status.

71 Levine WC, Pope V, Bhoomkar A, Tambe P, Lewis JS, Zaidi AA, *et al.* Increase
• in endocervical CD4 lymphocytes among women with nonulcerative sexually transmitted diseases. J Infect Dis 1998; 177:167–174.
Attraction of lymphocytes by non-ulcerative sexually transmitted pathogens may be one factor for increased risk for HIV infection.

72 Cohen CR, Plummer FA, Hugo N, Maclean I, Shen C, Bukusi EA, *et al.* Increased interleukin-10 in the endocervical secretions of women with non-ulcerative sexually transmitted diseases: a mechanism for enhanced HIV-1 transmission? AIDS 1999; 13:327–332.

73 Hedges SR, Sibley DA, Mayo MS, Hook EW, Russell MW. Cytokine and antibody responses in women infected with *Neisseria gonorrhoeae*: effects of concomitant infections. J Infect Dis 1998; 178:742–751.

74 Alderete JF, Arroyo R, Lehker MW. Analysis for adhesins and specific cytoadhesion of *Trichomonas vaginalis*. Methods Enzymol 1995; 253:407–414.

75 Arroyo R, Engbring J, Alderete JF. Molecular basis of host epithelial cell recognition by *Trichomonas vaginalis*. Mol Microbiol 1992; 6:853–862.

76 Lehker MW, Arroyo R, Alderete JF. The regulation by iron of the synthesis of adhesins and cytoadherence levels in the protozoan *Trichomonas vaginalis*. J Exp Med 1991; 174:311–318.

77 Alderete JF, Engbring J, Lauriano CM, O'Brien JL. Only two of the *Trichomonas*
• *vaginalis* triplet AP51 adhesins are regulated by iron. Microb Pathog 1998; 24:1–16.
This investigation demonstrates for the first time that iron regulates virulence genes on the transcriptional level.

78 Engbring JA, Alderete JF. Three genes encode distinct AP33 proteins involved
• in *Trichomonas vaginalis* cytoadherence. Mol Microbiol 1998; 28:305–313.
This report describes the molecular characterization of a trichomonad adhesin protein and demonstrates the presence of multiple copies of the adhesin gene in the trichomonad genome.

79 O'Brien JL, Lauriano CM, Alderete JF. Molecular characterization of a third malic enzyme-like AP65 adhesin gene of *Trichomonas vaginalis*. Microb Pathog 1996; 20:335–349.

80 Engbring JA, O'Brien JL, Alderete JF. *Trichomonas vaginalis* adhesin proteins display molecular mimicry to metabolic enzymes. Adv Exp Med Biol 1996; 408:207–223.

81 Mirhaghani A, Warton A. Involvement of *Trichomonas vaginalis* surface-
• associated glycoconjugates in the parasite/target cell interaction. A quantitative electron microscopy study. Parasitol Res 1998; 84:374–381.
This study examines the possible role of glycoconjugates during cytoadherence. Binding to amnionic cell was established to involve carbohydrate interactions.

82 Singh BN, Lucas JJ, Beach DH, Shin ST, Gilbert RO. Adhesion of
• *Tritrichomonas foetus* to bovine vaginal epithelial cells. Infect Immun 1999; 67:3847–3854.
This report demonstrates that adherence of *T. foetus* is mediated by carbohydrate interactions. *T. vaginalis* adherence to vaginal epithelial cells, in contrast, does not involve carbohydrate structures.

83 Arroyo R, Gonzalez-Robles A, Martinez-Palomo A, Alderete JF. Signalling of *Trichomonas vaginalis* for amoeboid transformation and adhesion synthesis follows cytoadherence. Mol Microbiol 1993; 7:299–309.

84 Addis MF, Rappelli P, Delogu G, Carta F, Cappuccinelli P, Fiori PL. Cloning
• and molecular characterization of a cDNA clone coding for *Trichomonas vaginalis* alpha-actinin and intracellular localization of the protein. Infect Immun 1998; 66:4924–4931.
Actinin may play a major role in the morphological transformation of trichomonads during adherence. This study analyzed the cDNA sequence and its intracellular localization in the presence and absence of host cell signals.

85 Furtado MB, Benchimol M. Observation of membrane fusion on the interaction
• of *Trichomonas vaginalis* with human vaginal epithelial cells. Parasitol Res 1998; 84:213–220.
This study extends previous observations on the interaction of trichomonads with host cells. Extensive fusion of membrane was observed leading to lysis of the target cell.

86 Potamianos S, Mason PR, Read JS, Chikunguwo S. Lysis of erythrocytes by *Trichomonas vaginalis*. Biosci Rep 1992; 12:387–395.

87 Krieger JN, Ravdin JI, Rein MF. Contact-dependent cytopathogenic mechanisms of *Trichomonas vaginalis*. Infect Immun 1985; 50:778–786.

88 Dailey DC, Chang TH, Alderete JF. Characterization of *Trichomonas vaginalis* haemolysis. Parasitology 1990; 101:171–175.

89 Alderete JF, Garza GE. Specific nature of *Trichomonas vaginalis* parasitism of host cell surfaces. Infect Immun 1985; 50:701–708.

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

91 McGregor JA, French JI, Jones W, Parker R, Patterson E, Draper D. Association of cervicovaginal infections with increased vaginal fluid phospholipase A2 activity. Am J Obstet Gynecol 1992; 167:1588–1594.

92 Benchimol M, Batista C, De Souza W. Fibronectin- and laminin-mediated endocytic activity in the parasitic protozoa *Trichomonas vaginalis* and *Tritrichomonas foetus*. J Submicrosc Cytol Pathol 1990; 22:39–45.

93 Casta e Silva Filho F, de Souza W, Lopes JD. Presence of laminin-binding proteins in trichomonads and their role in adhesion. Proc Natl Acad Sci U S A 1988; 85:8042–8046.

94 Silva Filho FC, Ortega-Lopez J, Arroyo R. YIGSR is the preferential laminin-1
• residing adhesion sequence for *Trichomonas vaginalis*. Exp Parasitol 1998; 88:240–242.
This study established a specific laminin domain as the binding site for the trichomonad laminin receptor.

95 Lehker MW, Sweeney D. Trichomonad invasion of the mucous layer requires
• adhesins, mucinases and motility. Sex Transm Inf 1999; 75:231–238.
This report demonstrates and reinforces the importance of proteinases in trichomonad host interactions.

96 Buchvald D, Demes P, Gombosova A, Mraz P, Valent M, Stefanovic J. Vaginal leukocyte characteristics in urogenital trichomoniasis. Apmis 1992; 100:393–400.

97 Alderete JF, Kasmala L. Monoclonal antibody to a major glycoprotein immunogen mediates differential complement-independent lysis of *Trichomonas vaginalis*. Infect Immun 1986; 53:697–699.

98 Alderete JF, Provenzano D, Lehker MW. Iron mediates *Trichomonas vaginalis* resistance to complement lysis. Microb Pathog 1995; 19:93–103.

99 Caterina P, Lynch D, Ashman RB, Warton A, Papadimitriou JM. Complement-mediated regulation of *Trichomonas vaginalis* infection in mice. Exp Clin Immunogenet 1999; 16:107–116.

100 Rendon-Maldonado JG, Espinosa-Cantellano M, Gonzalez-Robles A, Martinez-
• Palomo A. *Trichomonas vaginalis*: in vitro phagocytosis of lactobacilli, vaginal epithelial cells, leukocytes, and erythrocytes. Exp Parasitol 1998; 89:241–250.

101 Hawes SE, Hillier SL, Benedetti J, Stevens CE, Koutsky LA, Wolner-Hanssen, Holmes KK. Hydrogen peroxide-producing lactobacilli and acquisition of vaginal infections. J Infect Dis 1996; 174:1058–1063.

102 Min DY, Ryu JS, Park SY, Shin MH, Cho WY. Degradation of human immunoglobulins and cytotoxicity on HeLa cells by live *Trichomonas vaginalis*. Korean J Parasitol 1997; 35:39–46.

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

104 Wang A, Wang CC, Alderete JF. *Trichomonas vaginalis* phenotypic variation occurs only among trichomonads infected with the double-stranded RNA virus. J Exp Med 1987; 166:142–150.

105 Alderete JF. *Trichomonas vaginalis* NYH286 phenotypic variation may be coordinated for a repertoire of trichomonad surface immunogens. Infect Immun 1987; 55:1957–1962.

106 Alderete JF, Kasmala L, Metcalfe E, Garza GE. Phenotypic variation and diversity among *Trichomonas vaginalis* isolates and correlation of phenotype with trichomonal virulence determinants. Infect Immun 1986; 53:285–293.

107 Alderete JF. The *Trichomonas vaginalis* phenotypically varying P270 immuno-
• gen is highly conserved except for numbers of repeated elements. Microb Pathog 1999; 27:93–104.
This study demonstrates that the DREGRG epitope of P270 is present as multiple repeats with the p270 gene. Size polymorphism of the protein is due to the number of repeats present in the gene.

108 Alderete JF. Iron modulates phenotypic variation and phosphorylation of P270 in
• double-stranded RNA virus-infected *Trichomonas vaginalis*. Infect Immun 1999; 67:4298–4302.
The importance of this study is that it suggests that iron uptake may be linked to signal transduction pathways involving protein phosphorylation.

109 Lehker MW, Alderete JF. Properties of *Trichomonas vaginalis* grown under chemostat controlled growth conditions. Genitourin Med 1990; 66:193–199.

110 Lehker MW, Alderete JF. Iron regulates growth of *Trichomonas vaginalis* and the expression of immunogenic trichomonad proteins. Mol Microbiol 1992; 6:123–132.

111 Kostara I, Carageorgiou H, Varonos D, Tzannetis S. Growth and survival of *Trichomonas vaginalis*. J Med Microbiol 1998; 47:555–560.

112 ter Kuile BH, Bonilla Y. Influence of growth conditions on RNA levels in relation
• to activity of core metabolic enzymes in the parasitic protists *Trypanosoma brucei* and *Trichomonas vaginalis*. Microbiology 1999; 145:755–765.
This report represents a systematic examination of coordinated regulation of metabolic enzymes in response to environmental variables. The study suggest that trichomonad metabolic enzymes are regulated at the post-transcriptional level.

113 Fast NM, Doolittle WF. *Trichomonas vaginalis* possesses a gene encoding the
•• essential spliceosomal component, PRP8. Mol Biochem Parasitol 1999; 99:275–278.
An important study raising the possibility of trans-splicing in trichomonads.

114 Liston DR, Johnson PJ. Analysis of a ubiquitous promoter element in a primitive
•• eukaryote: early evolution of the initiator element. Mol Cell Biol 1999; 19:2380–2388.
The first study to describe a core promoter element for trichomonad genes. The promoter element is most homologous to the metazoan initiator element, but in contrast to metazoan appears to be ubiquitous in trichomonads.

115 Lehker MW, Alderete JF. Resolution of six chromosomes of *Trichomonas*
• *vaginalis* and conversation of size and number among isolates. J Parasitol 1999; 85:976–979.
This report describes for the first time the conditions for the electrophoretic separation of trichomonad chromosomes. Large chromosomal rearrangements do not appear to be involved in the heterogeneity of trichomonads.

116 Schneider A, Plessmann U, Felleisen R, Weber K. Posttranslational modifications of trichomonad tubulins; identification of multiple glutamylation sites. FEBS Lett 1998; 429:399–402.

117 Zuo Y, Riley DE, Krieger JN. Flagellar duplication and migration during the
• *Trichomonas vaginalis* cell cycle. J Parasitol 1999; 85:203–207.
This study describes the behavior of flagella during the cell cycle and concludes that trichomonad flagella appear to behave like centrioles during S-phase.

118 Katiyar SK, Visvesvara GS, Edlind TD. Comparisons of ribosomal RNA sequences from amitochondrial protozoa: implications for processing, mRNA binding and paromomycin susceptibility. Gene 1995; 152:27–33.

119 Embley TM, Hirt RP. Early branching eukaryotes? Curr Opin Genet Dev 1998; 8:624–629.

120 Germot A, Philippe H. Critical analysis of eukaryotic phylogeny: a case study
• based on the HSP70 family. J Eukaryot Microbiol 1999; 46:116–124.
HSP70 sequence analysis was unable to generate robust interphyla relationships among the early branching eukaryotes. This analysis supports the concept of a very rapid diversification of eukaryotes.

121 Viscogliosi E, Muller M. Phylogenetic relationships of the glycolytic enzyme,
• glyceraldehyde-3-phosphate dehydrogenase, from parabasalid flagellates. J Mol Evol 1998; 47:190–199.
This study demonstrates on the basis of sequence analysis of GAPDH that trichmonads use a GAPDH which is unique among all eukaryotes.

122 Roger AJ, Sandblom O, Doolittle WF, Philippe H. An evaluation of elongation
• factor 1 alpha as a phylogenetic marker for eukaryotes. Mol Biol Evol 1999; 16:218–233.
An important study showing that elongation factor 1 alpha is unable to resolve the phylogenetic relationships among early divergent eukaryotes.

123 Edgell DR, Malik SB, Doolittle WF. Evidence of independent gene duplications
• during the evolution of archaeal and eukaryotic family B DNA polymerases. Mol Biol Evol 1998; 15:1207–1217.
This report suggests that in early branching eukaryotes B DNA polymerases evolved by a series of gene duplications independent of gene duplications that gave rise to eukaryotic paralogs.

124 Peyretaillade E, Broussolle V, Peyret P, Metenier G, Gouy M, Vivares CP. Microsporidia, amitochondrial protists, possess a 70-kDa heat shock protein gene of mitochondrial evolutionary origin. Mol Biol Evol 1998; 15:683–689.

125 Karlin S, Brocchieri L. Heat shock protein 70 family: multiple sequence comparisons, function, and evolution. J Mol Evol 1998; 47:565–577.

126 Hashimoto T, Sanchez LB, Shirakura T, Muller M, Hasegawa M. Secondary
• absence of mitochondria in *Giardia lamblia* and *Trichomonas vaginalis* revealed by valyl-tRNA synthetase phylogeny. Proc Natl Acad Sci U S A 1998; 95:6860–6865.
This elegant study suggests, on the basis of the presence of valyl-tRNA synthase genes in all early branching eukaryotes, that premitochondrial species of eukaryotic origin do not exist among species living today.