Trichomonas vaginalis: paradigm of a successful sexually transmitted organism

S. RUGHOOPUTH and P. GREENWELL
School of Biosciences, University of Westminster, 115 New Cavendish Street London W1W 6UW

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ABSTRACT

Trichomonas vaginalis (TV) is one of the most successful protozoan pathogens and one of the most common sexually transmitted organism in females, yet it is also one of the most poorly investigated. By producing a wide array of glycosidases and cysteine proteinase enzymes, the organism can easily adapt to the environment, harvesting host proteins and DNA for metabolism. With the ability to cause lesions, vaginitis and acute inflammatory disease of the genital mucosa, TV acts as a potential catalyst in the acquisition of secondary infections including human immunodeficiency virus (HIV) and human papillomavirus (HPV), the organism responsible for the pathogenesis of cervical cancer. Treatment of TV infection is relatively easy and could dramatically reduce the transmission of HIV in areas where TV is endemic.

KEY WORDS: Cervical cancer. Cysteine proteinases. Glycosidases. HIV. Trichomonas vaginalis.

Introduction

Trichomonas vaginalis (TV) is the most common agent responsible for sexually transmitted disease, with around 180 million new infections worldwide every year.[1,2] It is one of the least-well investigated infectious agents, yet one of the most prominent parasites. Trichomoniasis, the infection caused by *T. vaginalis*, is non-self limiting and gender discriminating as it infects mostly women. The classical clinical presentation is a profuse purulent malodorous vaginal discharge and pruritus. However, the infection can be subclinical or asymptomatic.[3] *T. vaginalis* is associated with punctate haemorrhage and inflammation of the cervix (strawberry cervix) that may mimic the cervical motion tenderness associated with pelvic inflammatory disease (PID).[4] Moreover, there has been recent evidence to implicate *T. vaginalis* in preterm delivery, low birthweight, infant mortality and predisposition to HIV and cervical cancer.[5] In men, infection often manifests as urethritis[6,7] and prostatitis.[8]

Despite being an infection mostly associated with women, a study by Krieger *et al*.[6] showed that 11% of men attending sexually transmitted disease (STD) clinics had *T. vaginalis* as the sole urethral pathogen, and more than 50% of these suffered from urethritis, implying a high association between infection and urethritis; however, trichomonads have been shown to be apathogenic in semen.[9] Most males are asymptomatic and therefore are unwittingly involved in the transmission of *T. vaginalis* to their partners. In this respect, the woman harbours the organism and the male acts as a vector, transmitting the disease from female to female.

Classically, diagnosis is dependent on the demonstration of organisms by direct microscopy of any discharge or by growth of *T. vaginalis* in a selective culture medium. These methods, used routinely in a diagnostic facility, have been successful in detecting the organism from swabs from female patients, but have been problematical when detecting *T. vaginalis* in male patients. Taking swabs from males can be painful or uncomfortable and the amount of material obtained is far less than that from female patients; hence, the numbers of *T. vaginalis* isolated are low, making detection difficult. However, the use of liquid-based cytology (LBC) as an alternative to conventional cervical scrapes is compatible with the detection of *T. vaginalis*.[10]

In 1999, Mahmoud and colleagues[11] described the use of a nested polymerase chain reaction (PCR) assay on DNA, isolated from vaginal discharge using a simple boiling method, for detection of *T. vaginalis*. In a cohort of 290 symptomatic patients with cervico-vaginitis and 160 asymptomatic women, they detected 35 positive samples using culture techniques. Of these, 12 (34.2% sensitivity) were positive by wet mount and 21 (60% sensitivity) by Papanicolaou smear. A one-tube nested PCR assay using primers targeting the Tv-E650 repeat sequence allowed them to detect all the culture-positive samples. Samples from patients with cervico-vaginitis not associated with *T. vaginalis* were negative in the PCR-based test.

Schwebke and Lawing[12] suggested that the use of culture techniques has shown a decrease in the prevalence of *T. vaginalis* amounting to 5%, while the use of PCR suggests that the prevalence has escalated by 17%. Their study further suggested that urine samples are more reliable than urethral swab as a source of *T. vaginalis* and concluded that sensitivity of PCR for *T. vaginalis* in urine samples was 100%. In 2003, Wendel and co-workers[13] utilised PCR to determine the prevalence of *T. vaginalis* in men attending STD clinics. In the same year, Schwebke and Hook[14] used a PCR-based approach to reappraise the prevalence of *T. vaginalis*, chlamydia and gonorrhoea in men attending STD clinics, and the results were 17%, 19.6% and 17.7%, respectively.

Correspondence to: Dr Pamela Greenwell
Email: greenwp@wmin.ac.uk

Crucitti *et al*.[15] also demonstrated that PCR is a more reliable method of detection than culture. Different primer sets gave sensitivities ranging from 60% to 90%. If probe-based detection of the amplicons is included post-PCR the sensitivity is improved. These authors analysed organisms in self-collected vaginal swab specimens and showed that PCR detection could be used in large population surveys, without the need for medical staff trained to take samples.

In a study in 2003, Lecke and colleagues[16] described the use of a fluorescence technique using Flutax, a fluorescent taxoid, to investigate the nature of microtubules in living protozoans. In the presence of *T. vaginalis*, the authors reported an intense fluorescence, and in their paper they discussed the diagnostic potential of Flutax.

Classification and structure

Donné first recognised the genus *Trichomonas* in 1837, and described it as a trophozoite with four free flagella, a costa at the base of the undulating membrane and an axostyle. Wenyon, in 1926, classified it in the family Trichomonadidae, characterised by the presence of three to five flagella, one axostyle protruding through the posterior of the cell, and a cytosome.

In 1947, Kirby regrouped TV into the class Trichomonadea of the order Trichomonadida, but subsequently Honingberg amended this in 1974. *T. vaginalis* was then described as having four to six flagella, which may be recurved, free or attached to an undulating membrane. Confusion about the number of flagella might be due to the fact that these workers were studying different trichomonads. These workers only described the trophozoite form and concluded that there was no cyst form in TV.

It is thought that the organism divides by binary fission, and is 7–32 μm long by 5–12 μm in width.[17] *T. vaginalis* can be oval to pear-shaped, but an amoeboid shape is evident in parasites adhering to mammalian cells,[18] with an undulating membrane along one side and four anterior flagella. A review of cell fractionation of parasitic protozoans by de Sousa and colleague[19] describes the fine detail of TV ultrastructure.

Antigenic characterisation

T. vaginalis has a conserved antigenic pattern and can be divided into two groups, which reflect the ability (or lack thereof) to secrete an antibody-binding surface protein. Although one group may not have the ability to express the protein on the cell surface, it nonetheless has the ability to synthesise the repertoire of immunogens.[20]

Type I can synthesise but does not undergo phenotypic variation for the surface expression of the immunogen, while type II is capable of phenotypic variation. Type II isolates can be further divided into three subpopulations. Type II TV is infected by a double-stranded RNA (dsRNA) virus (TVV) that causes the up-regulation, synthesis and surface expression of a highly immunogenic protein, P270.[21,22]

It is now clear that there is a correlation between the up-regulation of P270 at the transcriptional level and the presence of TVV. Additionally, the movement of P270 between the cytoplasm and the plasma membrane, which is an iron-regulated event, is seen only in TV isolates infected with TVV.[23] This virus, when present, affects the differential qualitative and quantitative expression of cysteine proteinases.[24] Patients infected with type I TV have subclinical infection or are asymptomatic, while those infected with type II TV often present with clinical manifestation of genital irritation and odour.[25]

Recently, Weber *et al*.[26] reported the presence of a dsRNA virus in 81.9% of the TV isolates they studied in South Africa. They confirmed the presence of this dsRNA virus by electron microscopy and nuclease digestion. In recent work carried out in our laboratory, we were also able to detect the presence of an extra RNA fragment during nucleic acid extraction. This fragment was approximately 4300 bp,[27] which is similar in size to the dsRNA virus.

Wendel and colleagues[25] reported that 65% of their type II cohort suffered from discharge, while 57% of their type I cohort suffered discharge. They also concluded that type II TV was more frequent in older women, while type I TV was more common in younger females. This finding may be due to the ability of the immune system to mount a more extensive immune response against the less virulent type I isolates.

T. vaginalis viruses

T. vaginalis viruses are dsRNA viruses that appear to exist in a number of different forms, even in a single protozoan cell. Whether these are different viral species or segmented viruses is still debated.[22,28–30] The sizes of the viruses vary between 4.3 and 5.5 kb.[31–33] Interestingly, there are reports of dsRNA satellites associated with TVV that resemble the mini-replicons reported to be associated with RNA viruses of yeasts and plants.[22] One satellite characterised possessed an open reading frame encoding an 8 kDa protein. Sequencing of TVV type 1 revealed the virus to be related to the Totiviridae that infect fungi and parasitic protozoans with respect to the organisation of its genome in which there were overlapping *cap* and *pol* genes.[28,29,34] These overlapping genes are translated into the capsid protein (CAP) and CAP-polymerase fusion protein (POL).[35]

Bessarab *et al*.[30] compared the sequences of two different TVV, TVV1-1 and TVV2-1, and showed that there was sequence conservation with respect to the *cap-pol* organisation. Additionally, they were able to show that TVV2-1 had two potential extra open reading frames compared to TVV1-1, suggesting the potential to synthesise additional proteins.

Snipes and colleagues[36] were able to demonstrate, using phylogenetic analysis, that there were two distinct lineages of TV that correlated with the presence of TVV. Indeed, the isolates infected with TVV were significantly more closely related than those without TVV. Non-parametric analyses also showed associations between resistance to metronidazole and the presence of TVV.

In 2002, Benchimol *et al*.[33] reported the ultrastructural analysis of a heterogeneous population of TVV found in a single TV isolate. The TVV were of different size (33–200 nm) and shape (filamentous, cylindrical and spherical). These results suggested that a single protozoan may be infected with different dsRNA viruses simultaneously and that TV could act as a reservoir for viruses.

The study also showed that viral particles were able to transfect virus-free TV. The mechanism of attachment appeared to be via endocyte-coated pits. The TVV were then found in the cells in vacuoles within the cytoplasm, and occasionally in the nucleus. The Golgi apparatus of infected cells showed changes in cisternae structure and electron density.

Exactly how TVV affect expression of P270 is not known. Indeed, it is not clear whether or not the presence of the virus affects expression of other TV proteins. The reported association of the presence of TVV with drug resistance is clearly an area of future research.

Hydrolytic enzymes

The presence of trichomonal proteinases has been known since the 1970s; however, interest in these has increased in the last decade. These proteinases vary in size[37] and those of lower molecular weight are released from the cells. These enzymes were observed in the culture supernatants during *in vitro* growth of TV[38] and in vaginal wash material from women with trichomoniasis.[39] However, there were quantitative and qualitative differences between proteinases among TV isolates. These proteinases have the ability to induce inflammation and therefore contribute to pathogenesis.

Lushbaugh and co-workers[40] reported that TV had a trypsin-like activity that caused detachment of eukaryotic (mammalian) cells in culture. These enzymes could degrade proteins such as laminin, virtonectin and other components of the extracellular matrix, influencing cell detachment from host tissues. Recent cloning studies have given credibility to this study, showing that there are four distinct cysteine proteinases. Amino acid sequence data indicate that these proteinases are of L-cathepsin and H-papain type.[41]

Connaris and Greenwell[42] demonstrated that both TV and *Tritrichomonas foetus* (a bovine pathogen) possess a wide range of intracellular glycosidases. On determining the activity of this group of enzymes, they found that β-galactosidase, α-N-acetylgalactosaminidase and β–N-acetylglucosaminidase are the most active. These enzymes have the ability to metabolise mucin, a high molecular weight glycoprotein comprising 80% carbohydrate, which constitutes the protective mucus layer of the female genital tract.[42]

Vella and Greenwell[9] successfully purified the enzyme β-galactosidase. Glycosidases also have the capacity to remove sugars from other cell surface and secreted glycoproteins, rendering them more susceptible to the actions of proteases.

It is likely that the glycosidases and proteases work in concert to degrade molecules such as immunoglobulins targeted towards the host and other organisms. In a study in our laboratory, we were able to show that TV can degrade IgG, IgM and IgA and hence have the capacity to remove functional immunoglobulins from the infected urinogenital tract. Therefore, by compromising the immune response, TV is a facilitator for other sexually transmitted diseases.

Connaris[43] demonstrated that *T. foetus*, the related bovine parasite, is able to control the pH of its environment. In experiments designed to determine the effect of different pH values on organism survival and metabolism, it was observed that cultures buffered between pH 5 and pH 9 all had a pH of 6 after two days' growth. This implies that *T. foetus*, and probably TV, has a mechanism for both raising and lowering the pH of the microenvironment. However, organisms began to die below pH 5.

Adherence

Trichomonas vaginalis is highly evolved and capable of surviving in an adverse host environment. To be able to survive in the female genital tract, this organism must overcome the fluid flow barrier, infiltrate the mucus bathing the vaginal epithelial cells, maintain a micro-environment of the appropriate pH and have the ability to evade the immune system. Most importantly it must grow and multiply within the host environment with nutritional limitation.

Thus, as a mechanism of evolution, TV have become mucosal parasites, penetrating the mucous layer to parasitise the vaginal epithelium. To prevent being washed away by the constant vaginal secretions, TV must adhere to the epithelium.

Alderete and Garza[20] analysed the mechanism of adhesion of TV to epithelium and determined the nature of the adhesion molecules. They reported that trypsinisation of live organisms prevented cytoadherence and they concluded that there was a surface protein that mediated host parasitism. Further work showed that there were four trichomonad proteins responsible for cytoadherence to vaginal epithelium. Other studies suggested that the enzyme cysteine proteinase was responsible for trichomonal cytoadherence and cysteine proteinase inhibitors were used successfully to reduce cytoadherence by 80%.

One essential element for successful growth of TV is iron, which favours competition between pathogens and is important in the development of virulence properties. Iron plays a paramount role in cytoadherence and studies have shown that excess iron in the growth medium encourages attachment of trichomonads to the epithelium. Trichomonads therefore have the ability to infect menstruating females, where blood provides an abundance of iron, encouraging adherence of the organisms and resulting in a higher propagation rate.

The proteins AP65, AP51, AP33 and AP23 are synthesised in TV in the presence of iron. Garcia and co-workers[44] described the increase in surface-expressed adhesins on contact of TV with the host epithelium. They also determined that there was a direct relationship between adherence and the amount of adhesin bound. Interestingly, they described a TV isolate, MR100, that did not synthesise adhesins and was non-adherent, reinforcing the role of adhesins in attachment of TV.

AP65, AP51, AP33 and AP23 are expressed on the TV cell surface and interact with the host epithelial cells by ligand receptor-type interactions. Recombinant forms of these adhesins act as competitive inhibitors of TV binding to HeLa cells in culture.[23] Alderete's group has reviewed the apparent enzyme activity of adhesin molecules and summarised the evidence that adhesins, when secreted, appear to act as enzymes.[44–45] This work suggests the possibility that sugar hydrolases act as lectins on cell surfaces. This phenomenon has been described as a function of the β-N-acetylglucosaminidase in mammalian sperm that acts as a lectin in fertilisation.[46]

Metabolic requirements

Trichomonads cannot synthesise purines and pyrimidines *de novo*. Indeed, purine-ring precursors are not incorporated into nucleic acids; however, the organism directly salvages purine nucleosides through adenosine and guanosine kinases. There is no incorporation of inosine or hypoxanthine and no inter-conversion between adenylate and guanylate.[47]

Nucleotides and not nucleosides are transported across the TV membrane. The nucleotides are transported via the membrane by a two carrier-facilitated transport mechanism at a rate sufficient to sustain growth.[48] The nucleotides are then converted to nucleosides intracellularly. The organism lacks enzymes required to synthesise DNA *de novo*, and therefore must have quick access to DNA as a source of nucleotides before replication can occur.[42] This is achieved via physical contact and lysis of the human cells or by degradation of fungal, microbial or leucocyte DNA. It has been shown that TV secrete a potent DNase that completely degrades human, fungal, yeast and bacterial DNA, although they do not appear to have enzymes that degrade RNA.

Most of the energy metabolism is anaerobic. Work carried out by Lindmark and Müller[49] showed that the organisms possess a respiratory organelle termed a hydrogenosome, which appears as a chromatic granule by light microscopy. These are double membrane-bound organelles associated with catabolic process-extending glycolysis.[50] Malate is converted to pyruvate by NAD- dependent malic enzymes. The pyruvate then undergoes oxidative decarboxylation catalysed by the enzyme pyruvate ferrodoxin oxidoreductase through a ferredoxin-mediated electron transport system.

During this process, molecular hydrogen is produced in the hydrogenosome, hence the name. As this organelle is responsible for fermentative carbohydrate metabolism, it is probably analogous to the mitochondria of eukaryotes, as studies have shown that TV lack mitochondria. These organelles, however, do not contain DNA.[51]

Hydrogenosomes are a target for the antiprotozoal drug metronidazole and derivatives of 5-nitroimidazole. The drug is metabolically activated, resulting in the release of reactive nitro anion radicals. This is made possible by the fact that metronidazole acts as a preferential electron acceptor.

The hydrogenosomal enzyme, pyruvate ferredoxin oxidoreductase (PFOR), catalyses the oxidative decarboxylation of pyruvate to acetyl-CoA and CO_2.[52] Electrons are released in this reaction and are taken up by ferredoxin, a sulphur-iron protein that facilitates electron transfer in a range of biological systems.

The hydrogenase enzyme reoxidises the ferredoxin, forming molecular hydrogen as a result of electrons coupling with protons. If metronidazole is present, it competitively accepts the electrons, and this activation leads to reduced hydrogen production by the organelle.

When first introduced in 1959, metronidazole efficacy was nearly 95%, but resistance to this drug was reported in Canada within two years. This is also the case for the nitroimidazole derivatives. New DNA minor groove-binding drugs have been developed to overcome the problem of resistance.

These compounds, of which there are two classes, bind specifically to the minor groove of B-DNA (MGBs).[2] One class binds preferentially to GC sites by irreversible alkylation at the 2-amino group of guanines, while the other group binds reversibly at AT-rich regions of the minor groove, without major distortion to the DNA structure. Not only do these MGBs have antiprotozoal activity, they are also useful as antivirals, antibacterials and even antitumour agents.

One such drug that has proved useful against TV is a bisquaternary quinolinium compound, initially developed as an antileukaemic drug.[2] The knowledge of codon usage in TV has been exploited in this case to ensure specificity towards TV rather than the human host. The percentage of AT bases in TV is more than 70%, which is much higher than in the human host; therefore, these MGBs would preferentially bind to the trichomonal DNA.

Although TV undergoes anaerobic metabolism, sugars such as maltose and glucose are fermented by the parasite through a glycolytic pathway.[53] Ter Kuile and co-workers[54] suggested that glucose and maltose were a source of carbon and that glucose could be utilised through the Embden-Meyerhoff-Parnas pathway. Trichomonads lack both the cytochrome-mediated electron transport system and associated electron transport-linked phosphorylation; however, the transport of glucose via the membrane is by facilitated diffusion.

As trichomonads do not have the ability to synthesis lipids *de novo*, they have acquired specific mechanisms for lipid acquisition. Studies have demonstrated that there is flexibility in the lipid composition of the membrane. This variation is a response to the vaginal conditions at colonisation.

One study showed that TV and *T. foetus* contained five to 17 times more glycosphingolipids than the medium,[55] demonstrating the ability of trichomonads to assemble complex glycolipids from precursor molecules. Beach and colleagues[55] even postulated that, as TV do not have enzymes capable of *de novo* lipid synthesis, they must have enzymes that can actively deacylate/reacylate phospholipids and hydrolyse exogenously obtained triacylglycerols. However, these enzymes have yet to be identified and characterised.[56]

Nutritional and pH limitations represent important environmental challenges that affect the protein-antigen composition of trichomonads. To resolve this problem and maintain antigenic heterogeneity, Tempest[57] grew the organism under conditions similar to the *in vivo* situation. This was achieved by continuous flow culture and thus the changes of physiological conditions observed could be correlated to the ability of the organism to cause infection.

Lehker and Alderete[58] found that the organism could be maintained in stable condition despite nutritional limitations. Trichomonads could achieve a generation time approaching 200 hours, which was in contrast to the four to six hours observed in organisms grown in batch culture. They found that pH affected cell density and it was possible to differentiate trichomonal isolates on the basis of survival at pH <5.

Recent conflicting studies have shown that TV are capable of regulating internal ionic balance and adapting to fluctuations in the human host. This mechanism is controlled by a P-type ATPase. This family of membrane proteins plays a role in cellular ion homeostasis. These ATPases share a highly conserved peptide motif containing an aspartate residue, are activated by Ca^{2+} and Mg^{2+} at different pH and are inhibited by vanadate.

Shah and co-workers[1] were able to identify eight members of the p-type ATPase family using a polymerase chain reaction (PCR)-based strategy. They designed primers that were able to recognise conserved motifs DKTGTLT for the ATP phosphorylation site and TGDGVND for the ATP binding site that are present in all p-type ATPases.

Following pairwise alignment, two groups of ATPase were revealed. Of these eight members, three were Ca^{2+} pumps, three were type IV phospholipid translocators, hence very important in the acquisition of lipids, a type-VIa SERCa (sarcoplasmic-endoplasmic reticulum calcium) pump and one type-V ATPase of unknown ionic specificity.

Trichomonads and respiratory infections

There have been several reports in the literature implicating TV in respiratory infections, especially in newborns.[59–61] In the case described by Hoffman *et al*.[59] in 2003 the neonate initially was diagnosed with urinary tract infection involving TV. However, the infection progressed to a cystic chronic lung disease suggestive of Wilson-Mikity syndrome. The authors speculated that the mother may have been infected and may have transmitted the infection during labour.

However, this was not true in the case described by Szarka and colleagues,[60] where the mother's clinical status was checked during pregnancy and found to be negative. Leucorrhoea was detected with changing intensity and TV infection was later diagnosed by culture. In all cases, the neonates had respiratory distress. It has been suggested that maternal TV infection may have resulted in an inflammatory response that triggered the respiratory distress.

Although respiratory TV infection in newborns is now well documented, there has been an increase in respiratory infections in immunocompromised patients, especially those who have acquired immune deficiency syndrome (AIDS).

Duboucher *et al*.[62] recently presented a case study of a 41-year-old man infected with human immunodeficiency virus (HIV) who was hospitalised with increasing dyspnoea and extensive ground-glass opacities on chest X-ray. Examination of bronchial washings showed the presence of trichomonads and *Pneumocystis* species. The trichomonads were later identified as TV by amplification and sequencing of the small subunit of the *rRNA* gene. The presence of TV in lungs of healthy or immunocompromised adults had not been described previously.

The authors suggest that trichomonads are overlooked parasites that may be regularly implicated in diverse human pathologies. Although an interesting finding, the presence of trichomonads in the lung is not surprising, as they contain large amounts of mucin, as is the case in the urinogenital tract, and dead or dying cells are also present, from which the protozoan could extract DNA, lipids and proteins.

Involvement of trichomonads in cervical cancer

In a cohort of 15,933 studied in Teipei by Wang and Lin,[63] the presence of candidiasis and trichomoniasis were highly associated with abnormal cytological findings and indicative of inflammation, but the authors failed to associate cervical cancer with trichomonad infection. In the same year, however, Zhang and co-workers,[64] in their study on a cohort of 16,797 from 1974 to 1985, found that TV infection contributed to the risk of cervical cancer.

In a cohort of 43,016 Norwegian women, studied between 1980 and 1989, a multiple regression model produced a relative ratio (RR; 95% confidence limit) of 2.1 for TV and 3.5 for HPV, demonstrating a correlation between TV and cervical neoplasia. Kharsany *et al*.[65] found the incidence of TV to be as high as 39% in women with cervical intraepithelial neoplasia (CIN). Upcroft and Upcroft demonstrated that TV predisposed to HIV and cervical cancer.[66]

Recent work by Sayed el-Ahl and co-workers[67] on 48 patients with invasive cervical cancer and 100 random age-matched female controls has shown that 19% and 5%, respectively, had antibodies to TV. These results suggest that, at some stage before the development of cancer, these patients had contact with TV. Patients with TV infection had a three-fold likelihood of contracting cervical cancer; hence, TV can be categorised as one of the most important co-factors in the pathogenesis of cervical cancer.

With reports of the emerging resistance of strains to metronidazole (the drug of choice for the treatment of TV infection), fewer patients clear infections and those that do not are susceptible to acquiring lesions, making the penetration of other sexually transmitted infections (STIs) easier.

Transmission of HIV

Trichomonas vaginalis is emerging as one of the most important co-factors in amplifying HIV transmission,[68,69] and it can also act as a portal for entry of HIV in an HIV-negative person. Studies from Africa suggest that TV infection can increase the rate of HIV transmission approximately two-fold. The mechanism for the transmission may be associated with genital ulceration, brought about by the various hydrolytic enzymes, including cysteine proteinases and glycosidases, secreted by trichomonads.[42] These would destroy host defensive proteins and glycoproteins, allowing access to underlying epithelium and the exposure of raw areas, facilitating viral entry.

In a cross-sectional study in four cities in sub-Saharan Africa, where two cities had a high prevalence of HIV and the other two a low incidence, Buve *et al*.[70] found that the prevalence of trichomoniasis was higher (mean: 31.8%) in the cities with high HIV prevalence, as opposed to the cities with low prevalence (mean: 10.4%). This work suggests that trichomoniasis may influence the spread of HIV. Indeed, work carried out by Weber *et al*.[26] may help to explain the high association between HIV and trichomoniasis, as 81.9% of their TV harboured TVV, which up-regulates the production of potentially cytotoxic cysteine proteinases.

Draper *et al*.[68] studied the effect of TV on secretory leucocyte protease inhibitor (SLPI) and found, not surprisingly, that these protective molecules present in vaginal secretions were rendered non-functional by the action of cysteine proteinases secreted by trichomonads. They concluded that degradation of these SLPI molecules might increase the risk of HIV acquisition.

A recent paper by Rendon-Maldonado and colleagues[71] demonstrated that HIV and HIV-infected lymphocytes can attach to TV in culture and are internalised via a mechanism

involving endocytic vesicles. The HIV particles may be harboured for a short time in TV but they are degraded in cytoplasmic vesicles within 48 hours. *Trichomonas vaginalis* also digested HIV-infected lymphocytes *in vitro*. However, as yet there is no evidence to suggest that TV can act as a viral vector.

Interactions with other organisms

At the University of Westminster, the interaction of TV with other STIs is being studied. In simple experiments it is easy to see the effect of co-culturing TV with bacteria or yeasts. Provided that the medium is not depleted by an overgrowth of the bacteria, TV will co-exist with lactobacilli, for example, and there is clear evidence that TV degrades the bacteria, as it quickly removes bacteria present at low density from cultures. Indeed, TV grows much better in the presence of low levels of bacteria.

Co-culturing *Candida albicans* and TV is also possible and both organisms grow well, although there appear to be exclusion zones around the TV into which the *C. albicans* will not grow. It is assumed that these zones represent a micro-environment containing hydrolytic enzymes. When *C. albicans* levels are low, TV will clear *C. albicans* infection completely. As TV does not inhabit a sterile environment devoid of other organisms, such interaction studies may lead to an understanding of the dynamics of urogenital infection.

Trichomonas vaginalis genome project

Recently Tang and co-workers at the Molecular Regulation and Bioinformatics Laboratory in Taiwan submitted more than 800 DNA sequences for TV, the result of a TV cloning and sequencing programme. Of the 860 sequences available through the NCBI website (www.ncbi.nlm.nih.gov/) only 181 show homology to any other nucleotide or protein sequence lodged in databases worldwide. Of these, few show any compelling homology to other genes.

A further 300 sequences derived from experimental data are also lodged in databases, although many of these are duplicate sequences from different laboratories, strains or sources of nucleic acid (mRNA, cDNA, DNA). However, it is interesting to note that most of the sequences derived from the TV sequencing project share no homology with other cloned and sequenced genes, implying that TV may have genes and proteins of novel structure, reflecting its place in evolution as one of the oldest eukaryote organisms.

The most current draft of the TV genome sequence can be found at www.tigr.org/tdb/e2k1/tvg/.

Discussion

Although Donné described TV in the early 1800s, it is only recently that interest in it has been shown by the scientific community. *Trichomonas vaginalis* remains one of the most common gender-discriminating sexually transmitted agents. It has a high social and economic impact, but has been cured effectively with metronidazole. However, with its proven association with HIV and cervical cancer, it will have a greater economic impact on developing countries, especially in sub-Saharan Africa where HIV is endemic.

Production of a plethora of enzymes makes TV one of the most adaptable organisms. It can easily maintain a niche, allowing it to thrive and infect new patients. Sadly, from a patient's perspective, the production of such enzymes acts to destroy protective factors and renders the host susceptible to viral infections such as HIV and probably HPV. Indeed, reports suggest a doubling of the risk of contracting HIV in patients with TV.[69] In a country where the prevalence of TV is 25%, the authors suggested that if TV infection amplifies transmission of HIV two-fold then 20% of heterosexual HIV transmission can be attributed to the presence of this trichomonad.

From an economic standpoint, treatment of trichomoniasis is cheap and readily available, whereas treatment for HIV is outside the reach of poor communities. Surely, therefore, diagnosis and treatment of TV infection are vital.

Clearly, an understanding of the pathogenicity of sexually transmitted organisms and their interactions should allow development of new diagnostic and treatment regimes. Sadly, discussions on sexually transmitted diseases, apart from HIV, are taboo. Due to the mode of transmission, there is a stigma attached to their acquisition and a reluctance both by government and individuals to discuss the scale of the problem. Nevertheless, through education and the development of new treatments, it may be possible to reduce levels of infection.

However, a recent report from the UK on the incidence of STIs is not encouraging. Even here, where there is access to education, confidential counselling and screening, there has been an upsurge in the number of cases of STIs reported.

In the quest to eradicate sexually transmitted diseases, including HIV, the UK Medical Research Council (MRC) should derive some benefit from Department for International Development funding of £16 million over the next five years. In the USA, in 2001, government agencies allocated $60 million for the development of appropriate microbicides,[72] which could provide a safe and undetectable barrier to infection.

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