New concepts in the diagnosis and pathogenesis of *Trichomonas vaginalis*

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R BHATT, M ABRAHAM, D PETRIN, GE GARBER. New concepts in the diagnosis and pathogenesis of *Trichomonas vaginalis*. *Can J Infect Dis* 1996;7(5):321-325. *Trichomonas vaginalis* infection is the most commonly encountered sexually transmitted disease. There is a need for more accurate and rapid laboratory diagnostic methods, leading to better control and treatment strategies. Various virulence factors such as adherence, contact-independent factors, hemolysis and acquisition of host macromolecules have been shown to play a role in the pathogenesis of this infection. Detection of the factors that are only present in the pathogenic isolates of trichomonads will lead to a better understanding of the epidemiology of this pathogen. Culture technique is highly specific compared with microscopic techniques, but it is time consuming. Immunological techniques lack proper correlation with clinical manifestations. The application of monoclonal antibodies, either singly or in a group that recognizes a common antigen, along with methods such as detection of common DNA fragment from clinical specimens, may have a promising future in the laboratory diagnosis of trichomoniasis.

Key Words: Cell detaching factor, Cysteine protease, *Trichomonas vaginalis*

Nouveaux concepts dans le diagnostic et la pathogenèse de l'infection à *Trichomonas vaginalis*

RÉSUMÉ : L'infection à *Trichomonas vaginalis* est la plus répandue des maladies transmises sexuellement. Il faut mettre au point des méthodes diagnostiques plus précises et plus rapides pour en arriver à des stratégies thérapeutiques et prophylactiques meilleures. Divers facteurs de virulence comme l'adhésivité, les facteurs indépendants du contact, l'hémolyse et l'acquisition des macromolécules de l'hôte ont un rôle important à jouer dans la pathogenèse de cette infection. Le dépistage des facteurs qui ne sont présents que dans les isolats pathogènes de *Trichomonas* amènera une meilleure compréhension de l'épidémiologie de ce pathogène. La technique de culture est très spécifique si on la compare aux techniques microscopiques, mais elle prend du temps. Les techniques immunologiques ne sont pas en très bonne corrélation avec les manifestations cliniques. L'application des anticorps monoclonaux, soit seuls ou en groupes, qui reconnaissent un antigène commun, de même que les méthodes comme le dépistage de fragment commun d'ADN à partir de spécimen cliniques pourraient receler d'importantes promesses pour le diagnostic de l'infection à *Trichomonas*.

Sexually transmitted diseases (STDs) constitute a group of infections that have long been an integral part of society. *Trichomonas vaginalis* infection is the most common STD (1,2). *T vaginalis* is a protozoan parasite that infects both men and women, especially during their sexually active years. In men, the infection is usually asymptomatic (3). In women, trichomoniasis exhibits a wider variety of symptoms than any other vaginal infection and the symptoms may range from

mild to severe; from the short-lived to the chronic; and from an isolated incident to the persistently recurrent (4). Complications due to trichomonal infection include adverse pregnancy outcome (5,6), low birth weight, postpartum endometritis (7), premature rupture of membranes (8), preterm delivery (9) and facilitation of human immunodeficiency virus (HIV) transmission (10). *T vaginalis* has adapted well to the continuously changing environment of the vagina. Recent research has

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Received for publication March 20, 1996. Accepted March 22, 1996

started to elucidate this dynamic interaction, helping to explain various aspects of its pathogenicity. These advances may lead to more sensitive techniques for diagnosis.

CULTURE AND SLIDE-BASED TECHNIQUES OF DIAGNOSIS

Direct wet-mount of fresh material and culture techniques are the most widely used techniques in diagnosis of trichomoniasis. Despite the advantage of immediate diagnosis by wet preparation, successful use of the method depends on the collection of the specimen, the amount of material on the swab, and the presence and the number of motile parasites. The sensitivity of wet preparation ranges from as low as 38% to as high as 82% (11). The insensitivity of the test is in part due to rapid loss of the characteristic motility by which this organism is identified and the presence of very few parasites. The wet preparation technique requires the presence of at least 10^4 organisms/mL to give positive results.

Various staining techniques such as Giemsa (12), acridine orange (13) and Papanicolaou (14) were introduced to improve the sensitivity of direct microscopy. However, staining techniques have their own limitations because the typical morphological characteristic of trichomonads are lost during fixation and staining steps. The inconsistency in the size and shape of the organisms, which on occasion may resemble polymorphonuclear leukocytes (which are present in abundance in vaginitis), adds to the difficulty in interpreting stained smears.

The broth culture method is described as the 'gold standard' for the diagnosis of trichomoniasis because it is simple to interpret and the technique detects the presence of comparatively very few organisms; however, it requires 48 to 72 h of incubation (15,16).

The cell culture technique was introduced by Hogue (17) in 1943 to study the cytopathic effect of this parasite. Since then numerous investigators have attempted to study this effect in various cell lines. Garber et al (18) used McCoy cells for the cultivation of clinical specimens of *T vaginalis* and showed it to be superior to wet preparation. They also showed that cell culture could assist in differentiating pathogenic isolates from nonpathogenic isolates. Kulda (19) and Alderete and Pearlman (20) offered support for this view when they observed that nonpathogenic isolates of trichomonad species failed to disrupt the cell culture monolayer. Most of the cell culture studies demonstrated that physical contact with cells was a major factor in the pathogenicity of *T vaginalis*. However, chemical secretable factors produced directly or indirectly (production of acidic metabolites by the living organisms can lead to hemolysis or death of cultured cells) (21) by the trichomonads have also been suggested as a pathogenic mechanism because cell-free filtrates have also showed similar cytopathic effect (22). Hence, the use of cell or tissue culture offers a means for analysis of the mechanisms of *T vaginalis* pathogenicity.

Diagnosis based on clinical presentation is neither sensitive nor specific, whereas wet preparation lacks sensitivity. The cell culture technique is not preferred because the pres-

ence of microbial flora in the vagina may interfere with the isolation and identification of the parasites. The use of selective media incorporating appropriate antibiotics to suppress vaginal flora is sensitive and specific and, consequently, is more sensitive than microscopic or other *in vivo* techniques. However, cell culture is expensive, not readily available, and is not amenable to rapid diagnosis. These limitations have prompted several attempts to detect circulating antibodies in the serum and secretory antibodies in urethral and vaginal exudate.

IMMUNE-BASED TECHNIQUES

Circulating antibodies to *T vaginalis* in infected patients have been demonstrated by agglutination (23), complement fixation (24), indirect hemagglutination, gel diffusion (25), fluorescent antibody (26) and ELISA (27). Most of these studies showed the presence of immunoglobulin (Ig) G rather than IgM in the sera of infected patients.

Using a mouse model of vaginal *T vaginalis* infection, protection from infection was demonstrated when the serum samples showed the presence of IgG class antibodies at a titre of 1:100 or more. This suggests a possible role of the humoral response in protection. However, humoral response has not been shown to protect humans. The role of cell-mediated response cannot be excluded because parasite killing independent of antibodies has also been observed (28-30).

The antibody response to the pathogen is influenced by various factors such as the nature of the antigen or pathogen, its live or inactivated form, inoculum size, and the frequency and length of exposure. Alderete et al (31,32) observed that all strains of *T vaginalis* synthesize but do not express certain high molecular weight proteins and only the trichomonads with the surface-exposed antigen induce an antibody response.

Cogne et al (33) used hemagglutination, immunofluorescence and ELISA to study the systemic antibody response to these parasites. They observed that ELISA showed better correlation with past and current infection and suggested its use as an alternative method in the diagnosis of infection.

Alderete (34) used ELISA to detect circulating antibodies by using whole cell and aqueous protein extracts as antigen. He also detected antibodies in patients with no previous or current history of infection. This could be due to the presence of natural antibodies. Bozner et al (35) showed that immunoprecipitation using protein A-bearing *Staphylococcus aureus* combined with electrophoresis in gelatin-polyacrylamide gels as a technique for demonstration of antibodies was superior to ELISA because no purification of *T vaginalis* antigen is required.

DNA TECHNIQUES

With recent advances in recombinant DNA technology, genes from pathogenic microorganisms have been cloned and used as probes for the detection of pathogens from clinical specimens. Wang and Wang (36) characterized the genome of *T vaginalis* and reported that it consists of approximately 2.5×10^7 base pairs with a high degree of repetitive sequences. This has been supported by the findings of Paces et al (37).

They showed that all isolates of *T vaginalis* had 10^2 to 10^5 repeats of Tv-E650 and used this in the detection of *T vaginalis* in clinical specimens.

Rubino et al (38) found a 2.3 Kb *T vaginalis* DNA fragment in all strains obtained from diverse geographic areas. They used this clone as a probe for the detection of *T vaginalis* DNA in vaginal exudates using a dot-blot hybridization technique. The probe was specific for *T vaginalis* DNA, did not react with other microbial flora of the vaginal tract, and was as sensitive as culture.

PATHOGENESIS OF *T VAGINALIS* INFECTION

Two schools of thought exist regarding the pathogenesis of *T vaginalis*, ie, contact-dependent (39) and contact-independent (21) mechanisms. It is likely that both are important. Graves and Gardner (40) showed that adherence, contact-independent factors, hemolysis, acquisition of host macromolecules by the organisms and the host response are all important factors in the pathogenicity of this parasite.

Four adhesin proteins ranging from 65 kDa to 21kDa or less, are associated with cytoadherence (40-42). These adhesins were not identified on *Thermoproteus tenax*, a non-pathogenic trichomonad. Protease treatment diminished cytoadherence, suggesting that these proteins are unique and important factors in the pathogenicity of *T vaginalis*. Furthermore, cysteine protease was found to be necessary for parasite adherence to epithelial cells. The adherence was inhibited when the parasites were treated with inhibitors of trichomonad cysteine protease. Similar findings were observed in our study using McCoy cell monolayer. When treated with N- α -p-tosyl-L-lysine chloromethyl ketone (TLCK), a cysteine protease inhibitor, the parasites failed to disrupt the monolayer for more than 8 h, whereas untreated parasites consistently disrupted the monolayer in under 5 h.

Coombs and North (43) and Lockwood et al (44,45) have shown the presence of multiple proteases in cell lysates of *T vaginalis*. Garber and Lemchuk-Favel (46), using an immunoblotting technique, have demonstrated the presence of two extracellular cysteine proteases (60 kDa and 30 kDa) from the cell-free filtrate of *T vaginalis*. The 60 kDa protease was produced by all 12 *T vaginalis* isolates used in the study. It was present in vaginal washes of mice and humans with active *T vaginalis* infection but absent in the vaginal washes of noninfected or successfully treated humans or mice. The detection of this factor, observed in pathogenic isolates only, may be a key step in studying pathogenesis and it may also serve as a diagnostic tool in active *T vaginalis* infection.

Krieger et al (47) reported that beta-hemolysin may be a virulence factor for *T vaginalis*. Many bacteria (48) and protozoa (49,50) have been shown to produce this virulence marker. Hemolysis may be important in providing nutrients from lysed erythrocytes because trichomoniasis is frequently exacerbated by menses. Fiori et al (51) and Arroyo et al (52) observed surface proteins in the range of 140 kDa to 33 kDa involved in hemolysis.

Contact-independent factors are also important in pathogenesis. Garber et al (22) demonstrated the presence of a 200

kDa contact-independent factor, a glycoprotein that causes monolayer cell detachment. This cell detaching factor (CDF) was observed in all 12 clinical isolates and a significant association of CDF production with clinical presentation was observed (53). The absence of this marker in *Pentatrichomonas hominis* suggests that it is a virulence marker. Many other studies also support a role of contact-independent cytotoxic mechanisms since the subepithelial vascularity seen in trichomoniasis does not always correlate with the number of parasites. Furthermore, supernatants from cultures of *T vaginalis* can cause a cytopathic effect in cell cultures (21,39).

The presence of these proteins in all the isolates of *T vaginalis* and in vivo experiments using a mouse model demonstrating that these proteins are immunogenic suggests that the proteins could be possible vaccine candidates or useful for diagnostic testing.

ROLE OF ANTIBODY IN PATHOGENESIS

T vaginalis possesses the ability to adsorb host plasma proteins that may have a function in nutrition or protection of the parasites. The host molecules may coat both the host and parasite components required for complement-mediated lysis. Specific local antibodies of IgA and IgG class have been identified in the cervicovaginal secretions of most infected individuals (54). Vaginal antitrichomonal antibodies have also been detected using the following techniques: ^{125} I-labelled antihuman IgA techniques (54), immunofluorescence (55) and ELISA (56).

IgA prevents adherence and subsequent uptake of pathogens by epithelial cells and it may potentiate opsonization of the parasites by IgG. However, Ackers et al (54) observed that antitrichomonas IgA was absent in the vaginal fluid of many patients with active *T vaginalis* infection, and Su (55) suggested that no correlation exists between the severity of the disease and IgG antibody level in the secretions. Using a mouse model, we were able to detect IgG and IgA antibodies in the vaginal washes of experimentally infected mice. This suggests that this model may be useful in studying various strategies to enhance the host immune response to infection.

Alderete (34) reported the presence of low titre IgA and IgG in vaginal secretions of infected females. This lack of correlation between antitrichomonal antibodies in vaginal washes and clinical picture of infection could be due to host plasma proteins that coat the parasites. This could influence the immune response and represent a mechanism by which the immune factors in the vagina are neutralized (34).

Extracellular protease production by *T vaginalis* may constitute a potential virulence factor by altering or inactivating a variety of host proteins. Protease production was observed in aerobic and anaerobic microorganisms found in the lower and upper genital tract sites of women with premature rupture of membrane and chorioamnionitis (58). Recently, Provenzano and Alderete (59) showed that *T vaginalis* is capable of degrading human immunoglobulins, and the presence of anti-protease antibodies in both serum and vaginal secretions of women was seen in active trichomoniasis.

Lockwood et al (45) and Garber and Lemchuk-Farel (60) found extracellular cysteine protease in culture medium and have suggested that these enzymes are also released from parasites in vivo. Proteases that degrade antibodies have been found in other protozoan parasites. Talbot et al (61) showed that *Trichomonas foetus*, a bovine trichomonad, releases extracellular cysteine protease that cleaves IgG class 1 and 2, along with other host proteins. Similar immunoglobulin degradation by *Streptococcus sanguis* and *Neisseria gonorrhoeae* was observed by Plaut et al (62). This could explain the possible role of protease in pathogenesis. Pathogenic trichomonad strains possess proteases that are required for adherence and the release of these proteases degrade immunoglobulins which can result in an alteration of the host immune response to infection.

In our preliminary experiments using a mouse model, we observed that cysteine proteinase is immunogenic in nature and that antibodies raised against this protease reacted with whole cell *T vaginalis* in a dot-blot immunoassay. We also observed that antiprotease antiserum could detect 10^5 *T vaginalis*/mL. This is more sensitive than the wet preparation. Bozner et al (35) also detected IgG specific for trichomonad cysteine protease in patients with trichomoniasis. This suggests that the detection of specific *T vaginalis* protease is a useful diagnostic tool.

Monoclonal antibodies to these immunogens could help to detect *T vaginalis* isolates from clinical specimens by using either a single or a group of monoclonal antibodies. Torian et al (63) prepared eight monoclonal antibodies of which one, directed against a 62 kDa polypeptide, reacted with all the isolates of *T vaginalis*. Krieger et al (15) obtained a group of nine monoclonal antibodies by using four isolates of *T vaginalis* as immunogens. *T vaginalis* isolates from the diverse geographic areas of North America reacted with at least one of the nine monoclonal antibodies. A pool of two broadly reactive monoclonal antibodies identified all 88 isolates tested.

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