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Antibody to *Trichomonas vaginalis* in Human Cervicovaginal Secretions

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Human cervicovaginal secretions were obtained from patients at the Gynecology and Obstetrics Clinics at National Taiwan University Hospital and Cathay General Hospital, Republic of China. Among the 500 patients examined, 33 (6.6%) were infected with *Trichomonas vaginalis* as determined by the culture method. Secretions from 24 of the infected patients and 30 noninfected women were assayed for anti-*T. vaginalis* immunoglobulins by the indirect immunofluorescent antibody technique. A few serum samples from both infected and noninfected persons were also included in this study. Immunoglobulin G (IgG) antibody against *T. vaginalis* was detected in 17 (70.8%) secretions from the infected women. Among the 17 positive secretions, anti-parasite IgA was found in two specimens, IgE was found in three, and IgM was found in one. Of the 30 secretions, 7 (23.3%) from noninfected women also contained anti-parasite IgG. Low levels of natural anti-trichomonad IgG and IgM were detected in the sera of normal persons. Infection with *T. vaginalis* caused an increase in the serum IgG antibody titer. Cross-reaction between *T. vaginalis* and *Pentatrichomonas hominis* was also observed.

Trichomonas vaginalis is a parasitic flagellate found in the urogenital tract of humans. Males harboring *T. vaginalis* may have acute or chronic urethritis or prostatitis, but ordinarily have no symptoms and may be unaware of the infection. Females may also harbor the parasite without any symptoms, but they usually have an increased vaginal discharge as well as itching and burning sensations of the genitalia (8, 15).

Circulating antibodies to *T. vaginalis* have been demonstrated in chronically infected patients by indirect hemagglutination (19), complement fixation (14), agglutination (24), and indirect immunofluorescence (17) tests. More recently Mason (18) reported that immunoglobulin G (IgG) antibody was more important in serum as ascertained by the indirect fluorescent antibody test, using fluorescein isothiocyanate-labeled anti-human IgM and IgG. An increased total humoral IgE level was also noticed in infected persons (9).

In studies of immunological response of the female reproductive tract, IgA, IgG, and IgM have been demonstrated in cervicovaginal secretions in certain microbial infections of the genital tract in both women and heifers (5, 6, 25). Chipperfield and Evans (4) noted an increase of IgA-, IgG-, and IgM-containing plasma cells in endocervical tissues from women with gonorrhea, trichomoniasis, and candidosis. Plasma cells of the IgM class, however, were more prominent in trichomoniasis than in the other two infections.

Attempts to demonstrate local antibodies in persons harboring *T. vaginalis* were unsuccessful (12) until Ackers et al. (1) demonstrated the presence of IgA antibody on the basis of radioimmunoassay by employing ^{125}I-labeled anti-human IgA. Bednova and Naftol'eva (2) also were able to detect antibodies to trichomonads by the immunofluorescence test in urogenital tract discharges of 25 out of 33 women with trichomoniasis, but the immunoglobulin class involved was not specified. This study was conducted to further identify the immunoglobulin classes of the anti-trichomonad antibodies in the cervicovaginal secretions of infected women by the indirect fluorescent antibody technique.

MATERIALS AND METHODS

Collection of specimens. Specimens were collected from patients attending the Gynecology and Obstetrics Clinics at National Taiwan University (Taita) Hospital and Cathay General Hospital, Republic of China. Cervicovaginal secretion of a patient was aspirated with a sterile Pasteur pipette and rubber suction bulb and then released into a test tube (13 by 100 mm) containing 0.5 ml of phosphate-buffered saline (PBS)

(0.04 M, pH 7.2). After specimen-containing tubes were brought to the laboratory, they were incubated at 37°C for 1 h before being centrifuged at 3,020 × *g* for 20 min at 4°C. The supernatants were then transferred into small vials and stored at −20°C. Specimens were numbered serially, and the name and history number of each patient were recorded for later examination of the patient's record.

Isolation and maintenance of *T. vaginalis*. At the time of collecting cervicovaginal secretion, vaginal material of the patient was also taken with a cotton swab for cultivation of *T. vaginalis* in Trypticase-yeast-maltose (TYM) medium (BBL Microbiology Systems) (7), pH 6.0, at 35°C. Axenic cultures were obtained with the aid of antibiotics (potassium penicillin G, 1,000 IU/ml; streptomycin sulfate, 1,000 μg/ml; kanamycin sulfate, 1,000 μg/ml) and then were examined microscopically daily for the presence of *T. vaginalis*. Cultures that were still negative after 7 days of incubation were considered negative and were discarded. All isolates were treated routinely three times with antibiotics. In subsequent transfers, no antibiotics and 5%, instead of 10%, inactivated horse serum was added to the medium.

The strains were assigned with serial numbers and cryopreserved in liquid nitrogen with 5% dimethyl sulfoxide as the cryoprotectant (13) after being maintained in vitro for 12 to 20 weeks.

Preparation of antigens. The four *T. vaginalis* strains used in this study were TH1, TH11, TH17, and TH24 (TH denotes Taita Hospital; the numeral represents the serial number of the isolate). These four strains were recovered from liquid nitrogen storage and transferred twice in TYM medium and then twice in TYM-NA (without agar) medium. Cultures 24 to 36 h old derived from the last transfer were centrifuged at 755 × *g* for 10 min and washed three times in Locke solution. Washed organisms were then fixed in 10% Formalin in Locke solution at 4°C for 24 to 48 h. Finally, the protozoa were washed three times and suspended in PBS (0.02 M, pH 7.5).

Eight circles, about 5 mm in diameter each and arranged in two rows, were marked with diamond pencil on a frosted-edge glass microscope slide (25 by 75 mm). The final trichomonad suspensions were delivered and spread onto each circle with a bacteriological loop. Smears were then dried on a slide-warming table adjusted to 41°C and stored at −40°C until needed.

A *Pentatrichomonas hominis* strain was kindly supplied by Hsin-Sheng Lo from the Department of Parasitology and Tropical Medicine, National Defense Medical Center, Republic of China, for checking the specificity of the indirect immunofluorescent antibody test. Antigen smears were prepared as above except that *P. hominis* was grown in modified TYI-S-33 (Trypticase, yeast extract, iron serum) medium (23).

Indirect fluorescent antibody technique. The fluorescein-conjugated IgG fractions of goat anti-human serum IgA (alpha chain specific, lot 13602), goat anti-human IgG (Fc fragment) (heavy chain specific, lot 13198), goat anti-human IgM (mu chain specific, lot 13383), and sheep anti-human IgE (heavy chain specific, lot 11918) were purchased from Cappel Laboratories. The specificity of each antiserum was checked by immunoelectrophoresis, using whole human serum as antigen, and the immunoelectropherograms generally were similar in pattern to the data sheets supplied by the manufacturer.

The slides were taken from storage, dried on the 41°C slide-warming table to eliminate condensation, and then rinsed in distilled water for 10 min to remove salt crystals. When dried, the smears of parasites were covered with 5 μl of cervicovaginal secretion or diluted serum sample and incubated in a moist chamber at 20°C for 30 min. Next, they were washed in PBS (0.02 M, pH 7.5) and distilled water for 30 and 10 min, respectively, and redried, and each circle was covered with 5 μl of fluorescein isothiocyanate-conjugated anti-human immunoglobulin. The conjugates were diluted 1:40, except in the case of anti-human IgG, for which a 1:80 dilution was used. After incubation at 20°C for 30 min, the slides were washed in PBS for 1 h before being washed for 10 min in distilled water. Finally, they were immersed in 0.1% aqueous Evans blue for 5 min and then washed in PBS and distilled water for 10 min each. After being dried, the antigen smears were mounted in buffered glycerol (9 parts glycerol to 1 part PBS, 0.02 M, pH 7.5). The slides were examined with a Leitz Orthoplan microscope equipped with an incident light fluorescence excitation system.

The cell fluorescence pattern was graded as follows: negative, the whole organism appeared red; 1+, the organisms exhibited weak green fluorescence, sometimes restricted to the periphery of the trichomonad while the inner part remained red; 2+, stronger fluorescence of the organism was observed, but red from the Evans blue counterstain was still visible; 3+, the whole organism emitted bright green fluorescence with little or no red from the counterstain.

Specificity of the assay system was also checked by adsorption experiment on three sera (no. 176, 252, and 433) from infected patients. Each of the sera (400 μl at 1:5 dilutions) was adsorbed with Formalin-fixed *T. vaginalis* (strain TH17) and *P. hominis*, respectively. The adsorbing organisms were prepared as per the procedure for affixing antigens to the slides. Sera with high IgG titers (no. 252 and 433) were adsorbed with 3 × 10^7 vaginal trichomonads and 4.5 × 10^7 intestinal trichomonads, respectively. The corresponding numbers of organisms used to adsorb serum with low IgG titer (no. 176) were 2 × 10^7 and 3 × 10^7, respectively. More *P. hominis* organisms were used in the adsorption experiments to compensate for their smaller size as compared with *T. vaginalis*. The serum-antigen suspensions were incubated in a 37°C water bath for 2 h with agitation every 15 min. Adsorbed sera were recovered by centrifugation at 4,340 × *g* for 15 min at 4°C and stored at −60°C until needed.

RESULTS

Among the 500 women examined, 33 (6.6%) had *T. vaginalis* infection as ascertained by the culture method. Another patient whose infection was not detected by the culture method was diagnosed by the Papanicolaou smear. Samples that were contaminated with blood or contained scanty secretions were excluded from the fluorescent antibody assays. Secretions from 24 infected patients and 30 noninfected women (as ascertained by the culture method) were assayed

TABLE 1. Antibodies to *T. vaginalis* in the cervicovaginal secretions and sera of seven infected patients

Serum no.	Fluorescence[a] (cervicovaginal secretion)				Serum Ab titer (reciprocal of dilution)				Time lapse[c] (wk)
	IgA	IgE	IgG	IgM	IgA	IgE	IgG	IgM	
252	1+	1+	3+	1+	5	32	1,000 (200)[b]	10	1
433	–	–	3+	–	5	<5	1,000 (100)	10	4
240	–	–	1+	–	<5	<5	100 (40)	10	0
209	–	–	–	–	<5	5	200 (40)	10	2
342	–	–	–	–	<5	<5	100 (40)	10	1
176	–	–	–	–	<5	<5	100 (40)	20	0
162	–	–	–	–	<5	<5	100 (40)	5	1

[a] See the text. —, negative reaction.
[b] Antibody titer in reaction with *P. hominis* antigen is shown within parentheses.
[c] Difference in time between date of cervicovaginal secretion collection and blood withdrawal; zero indicates that both samples were taken on the same day.

for antibodies to *T. vaginalis* by the fluorescent antibody technique.

Antibody to *T. vaginalis* belonging to the IgG class was detected in 17 (70.8%) of the 24 secretions from infected patients. Among the 17 specimens, a few secretions also had low level of anti-trichomonad antibody of other immunoglobulin classes. Specific IgA was demonstrable in two specimens (no. 132 and 252), IgE in three (no. 252, 412, and 464), and IgM in one (no. 252). All four secretions contained a relatively high level of IgG antibody against *T. vaginalis*. In the control group, anti-trichomonad IgG was detected in seven (23.3%) specimens, but no antibody belonging to the other three classes were detectable by the method used in this study.

No attempt was made to titrate the antibody titer of the cervicovaginal secretions that exhibited positive reactions because the initial dilution factor was not measured. However, it is interesting to note that among the 17 anti-trichomonad IgG-containing secretions from infected women, about one-half (7/17) resulted in weak fluorescence (1+), whereas in the control group, most of the positive reactions (6/7) had relatively stronger fluorescence (2+ or 3+). No correlation was found between the level of antibody in the secretions and severity of the infection. Only a slight difference in the degree of fluorescence was observed in reactions of the four *T. vaginalis* strains with a few of the antibody-containing secretions.

Blood was withdrawn from seven of the infected patients, either on the day of cervicovaginal secretion collection or 1 to 4 weeks thereafter. Control sera were obtained from eight noninfected adults and seven children under 10 years old. Serial dilutions of the inactivated sera were used in the assays. No IgA or IgE antibodies to *T. vaginalis* were detected in the control sera at dilutions of 1:4 and above. Low levels of anti-trichomonad IgM and IgG were present in the control sera (antibody titers: IgM, 1:5 to 1:20; IgG, 1:10 to 1:40). There was no significant increase in IgM level in the sera from infected patients, but a small increase in IgG titer (up to 1:100 to 1:200) was observed in most of the samples. The antibody level in the cervicovaginal secretions of these patients was low or undetectable. A very high increase in serum IgG titer (up to 1:1,000) was observed in two patient samples in which the cervicovaginal secretions also contained a relatively high concentration of anti-parasite IgG (Table 1). However, no precipitin line was observed in double diffusion tests, using serum or cervicovaginal secretions from these patients in reaction with strain TH17 homogenate. Low levels of anti-*T. vaginalis* IgA and IgE were also present in a couple of sera from infected patients (Table 1).

TABLE 2. Results of adsorption experiment

Serum no.	Adsorption with organism:	IgG titer tested with:	
		TH17	*P. hominis*
176	TH17	<5	10
	P. hominis	20	<5
252	TH17	10	20
	P. hominis	160	5
433	TH17	5	40
	P. hominis	80	5

To check the specificity of the test, antigen smears of *P. hominis* were treated with some of the cervicovaginal secretions and all of the sera. Sixteen cervicovaginal secretion samples were taken from each group (infected versus control) for assay. Cell fluorescence patterns resulting from reactions of *P. hominis* to secretions from women having IgG to *T. vaginalis* were usually one or two grades lower than those of homologous reactions. As in the case with *T. vaginalis*, control sera also contained low levels of IgM and IgG to *P. hominis* (antibody titers, 1:10 to 1:40). Moreover, infection with vaginal trichomonad did not change the IgM titer. The IgG levels in sera from these patients were mostly within the range of control sera, but the two sera that had high IgG titers to *T. vaginalis* also showed a significant, although much lower, increase in IgG titer to *P. hominis* (Table 1). Results of the adsorption experiment (Table 2) show that vaginal trichomonads were more effective than intestinal trichomonads in reducing anti-*T. vaginalis* IgG titers of sera. On the other hand, intestinal trichomonads were more effective than vaginal trichomonads in reducing the anti-*P. hominis* IgG titer of the tested sera. These results indicate that common antigenic determinants are shared by vaginal and intestinal trichomonads, but each species also has its specific antigens.

Another interesting phenomenon is that some of the human cervicovaginal secretions may cause lysis of the trichomonads. In preliminary tests, TH1 strain organisms were fixed and kept in 1% Formalin for about 1 month before being affixed to glass slides. Of the 24 secretions, 15 from infected patients caused various degrees of damage to cell membranes; some resulted in slight lysis, whereas others resulted in complete lysis of the trichomonads. Inactivation of secretions at 56°C for 30 min did not destroy the lytic factor in most secretions; therefore, the complement system may not be responsible for lytic action. Occurrence of the lytic factor does not seem to be related to anti-trichomonad activity because, among the 15 samples from infected women, 5 had no detectable antibodies, 3 had a 1+ reaction, 3 were 2+, and 4 were 3+. In the control group, all eight secretions that caused cell lysis were antibody negative. Although the exact substance that caused cell lysis was not known, it is interesting to note that more infected than control group secretions contained this lytic factor (62.5 versus 26.7%). In later experiments, the organisms were fixed in 10% Formalin, and the lysis phenomenon was reduced significantly, but five secretions from each group still caused varied damage to the fixed cells.

DISCUSSION

It is generally accepted that IgA is the prominent immunoglobulin in many external secretions in humans and other animals and that secretory IgA plays an important role in immunity to certain viral and bacterial infections of the respiratory and gastrointestinal tracts (10). Relatively few studies have investigated the immunoglobulins in the cervicovaginal secretions during local infections (5). McMillan et al. (20, 21) reported that, in the course of gonorrhea infection, IgA and IgM antibodies were detectable in the serum of more than two-thirds of the women who had been infected for less than 2 weeks and that IgG antibody increased throughout the first week of infection and remained high for some time. In the cervicovaginal secretions of their patients, IgM antibody was similar to the serum antibody response, whereas IgG and IgA reactivities were detectable regardless of the duration of infection. In bovine vibriosis, however, it has been demonstrated that the vaginal IgA antibody response preceded the IgG response (6). Ackers et al. (1) demonstrated, by radioimmunoassay, the presence of anti-trichomonad IgA in the vaginal secretions in 76% of 29 infected and 42% of 19 apparently uninfected women. Unfortunately, results of their experiments with ^{125}I-labeled anti-human IgG serum were not included because the serum was not rendered heavy chain specific and cross-reacted with both IgA and IgM. Although they thought that low parasite counts in vaginal secretions might associate with the anti-trichomonad antibody, there is as yet no conclusive evidence that secretory IgA is related to immunity to *T. vaginalis* infection.

The primary aim of the present study was to identify the immunoglobulin classes of the anti-trichomonad antibodies in the cervicovaginal secretions of infected women. Unexpectedly, IgA antibody to *T. vaginalis* was demonstrated in only 8.3% of the cervicovaginal secretions from the 24 infected women, whereas IgG antibody was detectable in 70.8% of the secretions from these patients. Possible explanations for

the low rate of IgA detection in the cervicovaginal secretions in the present study as compared to that of Ackers et al. may be: (i) only a minute amount of IgA antibody is present in the cervicovaginal secretion that can be demonstrated by radioimmunoassay but not by the relatively less sensitive indirect fluorescent antibody technique; (ii) IgA may be produced by the host only during a certain period in the course of infection. The low IgA could be a result of the adsorption of the antibody by the organisms. It is also possible that *T. vaginalis* may be able to produce IgA proteases, as in the case with *Neisseria gonorrhoeae* and *Streptococcus sanguis* (22), so that IgA activity was reduced due to the presence of the enzymes.

Chipperfield and Evans (4) noted a prominent increase of IgA-, IgG-, and especially IgM-containing plasma cells in cervical tissues of trichomoniasis patients. However, in the present study, the IgM antibody was detected only in one of the secretions (4.2%) from 24 infected women. It is possible that, as in African trypanosomiasis, the parasite stimulated the production of nonspecific IgM, thus resulting in increased IgM-containing plasma cells in the cervical tissues observed by Chipperfield and Evans (4). IgM antibody production in trichomoniasis may also follow the pattern of antibody response in gonorrhea in that IgM was detectable mainly during the first 2 weeks of the infection (20, 21).

In the present study, IgG antibody against *T. vaginalis* was found in the cervicovaginal secretions of both infected (17/24) and noninfected (7/30) women. No correlation can be ascertained between the severity of the disease and the IgG antibody level in the secretion of the patient. Of the seven noninfected controls that had the antibody against the parasite, all had no symptoms suggestive of trichomoniasis on the day of sample collection. Examination of the patient records revealed that one patient had been treated for trichomonas vaginitis 14 years ago and she had subsequently been often bothered by leukorrhea. In another patient, the parasite was diagnosed in a wet smear preparation when she came to the clinics 40 days after the specimens were taken. The remaining five patients either came to the clinics for the first time or had no previous history of *T. vaginalis* infection. Studies by Teras and co-workers (12) revealed that complement fixation antibody and agglutinin titer dropped gradually after metronidazole treatment, and, in the majority of cases, the antibodies disappeared from the serum within 1 year after the cure. They also pointed out that positive reactions in apparently parasite-free people may indicate the presence of cryptic infections or the presence of antibodies left from previous infections. Although 14 years seemed a bit too long, the presence of antibody in the cervicovaginal secretions of apparently parasite-free women still may be related to a latent infection or previous infection. Of course, the possibility of a false-positive reaction due to *P. hominis*, *T. tenax*, or other cross-reacting organisms also cannot be ruled out.

It has been reported many times that natural antibodies against *T. vaginalis* existed in normal human serum (11) and that common antigens existed between the vaginal and the intestinal trichomonads (3, 16). The present study substantiated these findings; however, it was further demonstrated that normal human serum also contained natural antibodies to *P. hominis* and that the natural antibodies against the two trichomonads included both IgG and IgM. Although only a limited number of sera were available for the immunofluorescence assay, a significant increase in anti-trichomonad IgG titer was observed among the infected patients when compared with that of the control group. This agrees with Mason's (18) finding that IgG, rather than IgM, appeared to be the anti-trichomonad antibody in the sera of infected patients.

The origin of the IgG antibody in the cervicovaginal secretions of infected patients is an interesting question. Further studies are needed to verify whether it is synthesized locally or results from transudation of the serum antibody and also whether the cervicovaginal anti-trichomonad IgG plays any role in immunity to reinfection or pathogenesis of the disease.

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