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Detection and Characterization of Serum Antitrichomonal Antibodies in Urogenital Trichomoniasis

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Antibodies against *Trichomonas vaginalis* were detected in serum samples from 98 patients by three immunological assays. A good correlation was observed between the enzyme-linked immunosorbent assay and the immunofluorescence method, whereas it was found that the enzyme-linked immunosorbent assay correlated better with the current or past detection of organisms than did other serological methods (immunofluorescence and hemagglutination). Similar results were obtained with whole trichomonads and two *T. vaginalis* soluble antigenic preparations, which suggests that immunodominant moieties shared by several *T. vaginalis* strains were detected. The level of antibodies of the immunoglobulin A class was higher in patients with past records of trichomoniasis, but less significantly so than the total antitrichomonal antibody level. Antibodies of the four immunoglobulin G subclasses were detected. Immunoglobulin G1 antibody values were higher in female than male patients.

Trichomoniasis is the most common infection of the urogenital tract. Trichomonal infection is recognized as a major, economically significant sexually transmitted disease. The need for a better understanding of the mechanisms involved in host-parasite relationships is underlined by recent reports of the refractiveness of some *Trichomonas vaginalis* infections to treatment with metronidazole (4, 8, 24) and the isolation of metronidazole-resistant strains (19, 20). A frothy discharge occurs in 12% of women with *T. vaginalis*, but local identification by microscopic wet-mount examination is not sensitive enough, as culture will detect twice as many *Trichomonas* infections (10). In addition, the presence of the parasite may remain undetected, especially in men, who often lack any clinical manifestation (9). This prompted several attempts at detecting circulating antibodies to the parasite (5, 12, 14–18, 25, 28, 30, 31).

In the present study, direct comparisons of the enzyme-linked immunosorbent assay (ELISA), immunofluorescence assay, and hemagglutination assay were made by using several antigenic preparations, which presumably contained antigenic moieties common to most *T. vaginalis* strains. We have characterized serum antibodies in 98 patients and 30 control individuals. In *T. vaginalis*-infected patients, data demonstrate the increase in the amount of serum antibodies directed to all antigenic preparations used and indicate that, as well as immunoglobulin A (IgA) and IgM antibodies, the four IgG subclasses are involved in human antitrichomonal antibody response.

MATERIALS AND METHODS

Patients. Serum samples from 98 patients (80 females and 18 males) with vaginitis or urethritis, or both, attending the Venereal Disease Outpatient Clinic of the Centre Hospitalier Universitaire of Rouen, France, were collected and frozen at −20°C until used. The ages of these patients ranged from 15 to 59 years (mean age, 29 years). At the same time, material for saline wet-mount examination, acridine orange-stained smears, and culture for *T. vaginalis* in agar-free TYM-Diamond medium (7) was obtained from the posterior vaginal fornix of women or from the urethral discharge of men. In 23 of these 98 patients, previous venereal infection had been reported (*Treponema pallidum*, *Neisseria gonorrhoeae*, *Haemophilus ducreyi* infection). In 14 of 98 patients, a *T. vaginalis* infection in the preceding months or years had been unambiguously demonstrated on smears.

Control sera for immunofluorescence and hemagglutination assays were obtained from 30 children under 8 years of age, who were admitted to a pediatric hospital for noninfectious conditions. For the ELISA, 20 additional control sera meeting the same requirement were studied (i.e., a total of 50 control sera for the ELISA).

Antigens. Six *T. vaginalis* strains isolated from the vaginal discharge of women attending the Rouen Venereal Outpatient Clinic were cultured in agar-free TYM-Diamond medium for 3 weeks. Two lines (X and Y) were prepared by mixing the axenic strains from two and four isolates, respectively. Cultures were kept under hydrogen and CO_2 in a jar and were passaged every 2 days for 1 month. The growth kinetics of each line were determined by counting viable cells at intervals: maximal growth rate was observed between 32 and 50 h of culture under the conditions used. The pathogenicity of these strains was determined in vivo (23) and in vitro (6).

Formalinized (10% formaldehyde, 5 min) X-line cells for immunofluorescence were obtained from 2-day cultures containing 70 to 80% viable cells. Soluble antigens for hemagglutination were prepared from the X *T. vaginalis* line by extraction with formamide by the procedure of Fuller (11). For sensitization, a 1% sheep erythrocyte suspension was mixed under agitation at 37°C for 60 min with an equal volume of antigen solution (16).

Soluble antigens for the ELISA were prepared as follows: X- and Y-line cells were washed in 0.001 M phosphate (pH 2)–0.14 M saline buffer (PBS) and subjected to ultrasonic treatment. Soluble antigen-containing supernatants were har-

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vested after centrifugation (23,000 × *g*, 4°C, 1 h) and dialyzed against water (20 h, 4°C). These soluble antigen preparations were termed antigen X and antigen Y, respectively. Whole cells for the ELISA consisted of washed parasites without further modification.

Immunofluorescence technique. Indirect fluorescence studies were performed as previously described (6). Briefly, sera were incubated on *T. vaginalis* smear spots for 30 min at 37°C. Slides were washed, air dried, and incubated with goat fluorescein-labeled anti-human immunoglobulin heavy and light chain antiserum (Institut Pasteur, Paris, France) (final dilution, 1/100). The slides were examined with an incident UV illumination microscope at a total magnification of ×200. Only marginal fluorescence of the body and flagella was considered positive; this excluded general fluorescence of the whole body of the organism. Results are expressed as the last positive serum dilution which labeled at least 50% of the trichomonads. Each experiment was done at least in triplicate.

Hemagglutination assay. Hemagglutination was performed as previously described (6). Samples (50 μl) of twofold serum dilutions in 0.85 M NaCl were distributed into the wells of round-bottom plastic microtiter plates, and 50 μl of a 1% suspension of sensitized sheep erythrocytes was added. The plates were gently agitated and incubated at 37°C for 1 h. Controls with sensitized and unsensitized erythrocytes and known negative and positive sera were included in each plate. The results were expressed as the last positive serum dilution. Each experiment was done at least in triplicate.

ELISA. For the ELISA, the following optimal conditions determined from preliminary experiments were used. For the *T. vaginalis* whole-cell antigen assay, microELISA plates (Dynatech, Plochingen, West Germany) containing 40,000 cells of the X line per well were desiccated in a drying oven at 37°C. The plates were then saturated with 200 μl of a 5% bovine serum albumin solution in PBS per well and maintained for 2 h at room temperature. The solution was decanted, and the wells were washed three times with cold PBS before use.

For the soluble antigen assay, plates were coated by incubating 200 μl of the X or Y antigen per well at concentrations of 160 and 185 μg of protein per ml, respectively (found to be within the optimal range in preliminary experiments), diluted in 0.1 M carbonate-bicarbonate buffer (pH 9.6) for 18 h at 4°C, washed, and filled with PBS-bovine serum albumin as described above.

For the estimation of total antitrichomonal antibodies, serial dilutions (1/200, 1/1,000, 1/5,000) of sera were performed in 0.5% bovine serum albumin–0.05% Tween 20–PBS. A 100-μl portion of each serum dilution was transferred in triplicate into antigen-coated microplate wells and incubated for 4 h at room temperature. The solutions were aspirated, and the wells were washed three times with cold PBS. Bound antibodies were detected with rabbit anti-human immunoglobulin beta-galactosidase-linked F(ab)'2 fragments (Amersham, England), the presence of which was revealed with *o*-nitrophenyl galactopyranoside. The results of the ELISA determinations were expressed as optical density at 405 nm (OD_{405}). ELISA determinations were performed at least in triplicate.

IgA antibodies were estimated in an ELISA with an alkaline phosphatase-linked anti-human IgA antiserum (TAGO, Burlingame, Calif.).

IgG subclasses of antitrichomonal antibodies were determined by a similar ELISA method with antigen X and specific monoclonal antibodies (Seward, England).

RESULTS

Comparison between immunofluorescence, hemagglutination and ELISA techniques for the determination of *T. vaginalis* serum antibody levels. In control sera, the optical density observed for a 1/200 serum dilution was less than 0.220 (mean, 0.171 + 0.040). We therefore considered as positive any determinations over a threshold of 0.290, i.e., 3 standard deviations from the mean of the controls. From immunofluorescence and hemagglutination data obtained in control sera, dilutions of 1/32 and 1/80, respectively, were found to be discriminative for antibody detection (data not shown).

Figures 1 through 3 show the distribution of ELISA, immunofluorescence, and hemagglutination antibody determinations in 98 sera from adult patients. A positive correlation was found between individual ELISA and immunofluorescence results ($r = 0.44$, $P < 0.01$). In contrast, no correlation was observed between hemagglutination and immunofluorescence or ELISA determinations ($P > 0.5$).

Comparison of whole cells and soluble *T. vaginalis* preparations as antigens in the ELISA. Figures 4 and 5 show that an excellent correlation was found between results obtained in the ELISA with whole cells and soluble X and Y antigens.

Relationships between serum antitrichomonal antibodies, parasite detection, and record of trichomoniasis. Among 80 female patients studied, 55 had antibodies detectable in the ELISA; this was the case for 7 of 18 male patients.

Table 1 shows the positivity or negativity of antibody detection in 98 sera from adult patients in relation to direct examination for *T. vaginalis* and previous history of trichomoniasis or other venereal disease. Among 25 patients

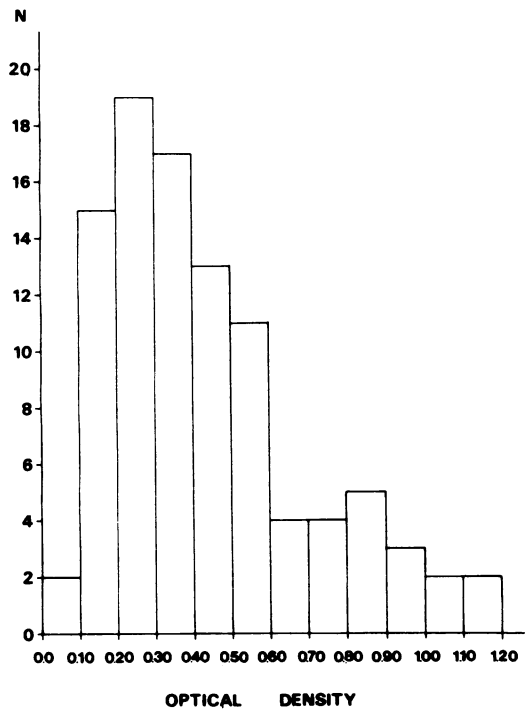

FIG. 1. Results of ELISA. Distribution of total antitrichomonal antibody levels in the sera of 98 patients. Results corresponding to 1:200 serum dilutions and expressed as the difference (OD_{405}) in serum minus the mean OD_{405} in control sera; i.e., 0.220. *N*, Number of patients.

(22 females, 3 males) harboring *T. vaginalis*, 24 (22 females, 2 males) and 20 (19 females, 1 male) had detectable antibodies by ELISA and immunofluorescence, respectively, versus only 16 (15 females, 1 male) by hemagglutination. In this group, serum from one male patient lacked any detectable antibody. Among the 14 patients with proven previous records of trichomoniasis and in whose sera no parasite could currently be detected, the ELISA was positive in 9 and immunofluorescence was positive in 6 (versus 3 only by hemagglutination). Lastly, among the 59 individuals without previous history of infection by *T. vaginalis* and whose sera were negative for current local parasite detection, two groups could be identified: 27 sera were negative by both ELISA and immunofluorescence (in that group, one patient reported a history of another venereal disease), whereas 32 were positive by ELISA or immunofluorescence (in that group, 13 patients reported a previous venereal disease). In these two groups, 13.5% of sera were positive by hemagglutination.

IgG subclass analysis of anti-*Trichomonas* antibodies. IgG1, IgG2, IgG3, and IgG4 antibody levels were simultaneously measured by the ELISA in 26 patients in whose serum antibodies had been detected (Fig. 6). The IgG1 antibody level was comparatively elevated in the female group (mean, 0.432 ± 0.069 versus 0.133 ± 0.035; $P < 0.05$). IgG2 levels were not significantly different (0.043 ± 0.009 and 0.050 ± 0.019, respectively). Although nonsignificant, IgG3 antibody levels were found to be higher in the male group (mean, 0.237 ± 0.120 versus 0.088 ± 0.023 in females). No difference was found for IgG4 levels (0.237 ± 0.068 in females, 0.218 ± 0.090 in males). The mean total antibody level was slightly higher in women, but this difference was not significant.

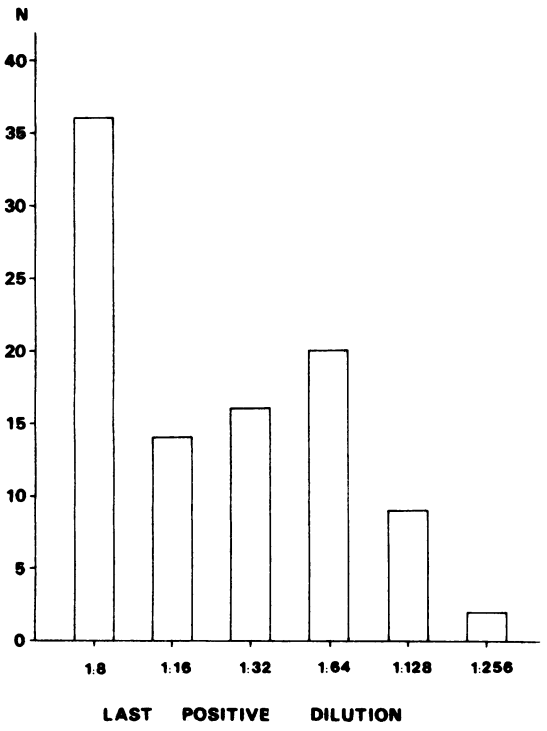

FIG. 2. Results of immunofluorescence assays. Distribution of total antitrichomonal antibody levels (same patients as for Fig. 1). Results are expressed as the last positive serum dilution. From data obtained in noninfected individuals, 1:32 was considered the first positive dilution.

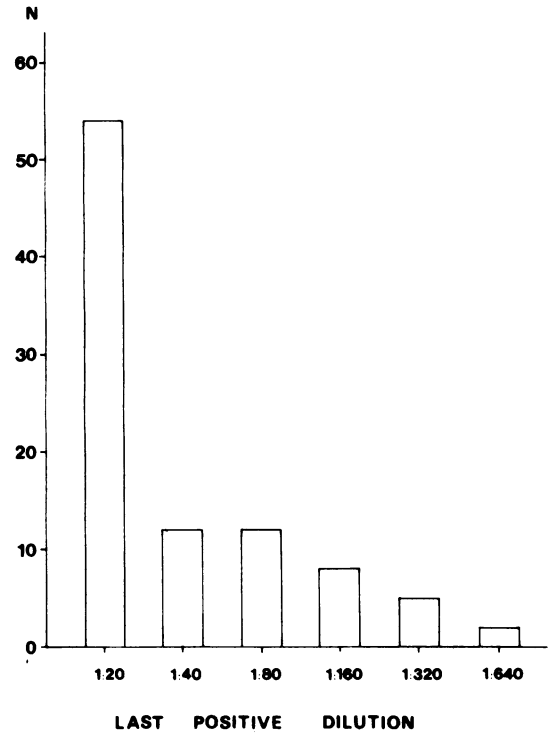

FIG. 3. Results of hemagglutination assay. Distribution of total antitrichomonal antibody levels (same patients as for Fig. 1). Results are expressed as the last positive serum dilution. From data obtained in noninfected individuals, 1:80 was considered the first positive dilution.

IgA antibodies. For currently infected patients, 85% of the sera had an IgA antibody level lying over 2 standard deviations of the mean of the control sera (i.e., 0.203 ± 0.043), whereas this percentage was 50 for patients with a past

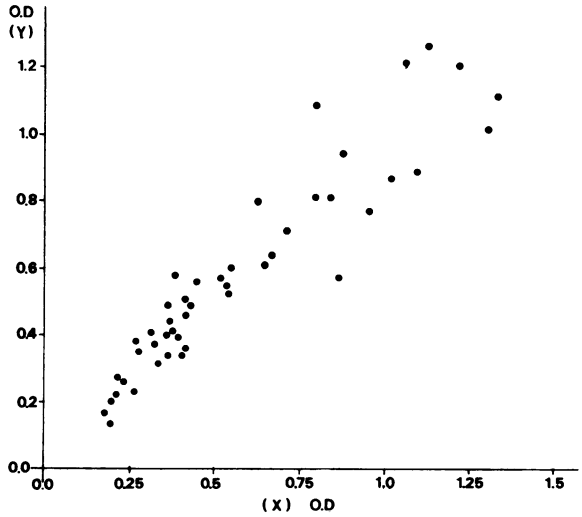

FIG. 4. Correlation between results obtained in ELISA assays with either X or Y soluble antigen. Results of total serum antibody levels in 48 patients were expressed as OD_{405} values corresponding to a 1:200 serum dilution ($r = 0.920$, $P < 0.01$).

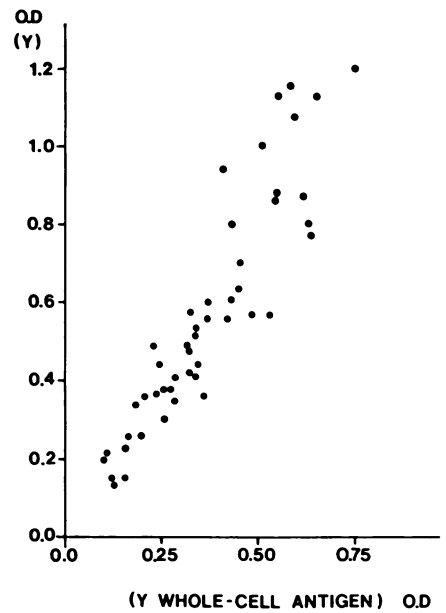

FIG. 5. Correlation between results obtained in ELISAs with either Y soluble antigen or whole cells from the Y *T. vaginalis* line. Results of total serum antibody levels in the same patients as in Fig. 4 were expressed as OD_{405} values corresponding to a 1:200 serum dilution ($r = 0.91$, $P < 0.01$).

record of trichomoniasis and 25 for patients without such a record. No difference was observed between positive sera from male and female patients (mean, 0.307 ± 0.046 and 0.327 ± 0.018, respectively).

DISCUSSION

The present study was aimed at characterizing the systemic antibody response to local infections by the parasite *T. vaginalis*. A sensitive ELISA method was developed by reference to well-established immunofluorescence and hemagglutination methods (5, 12, 15–18, 30, 31). The source of antigens consisted of organisms mixed in culture, providing antigen moieties common to most strains. In ELISA (as well as in whole-cell immunofluorescence), a significant background fixation was noticed with sera from unsensitized children, whatever the antigen used. This may be related to a passive membrane binding of plasma proteins, including human IgM and IgG, on a specific membrane trichomonal site (21, 22). A good correlation was observed between ELISA and immunofluorescence results with the whole parasite as well as with the soluble antigen preparations X and Y. This suggests not only that solubilization procedures did not alter the antigenic reactivity, but also that our soluble extracts contained immunodominant moieties shared by several *T. vaginalis* strains. This is in agreement with previous data obtained with human sera as well as with polyclonal or monoclonal antibodies from animals (3, 29). The correlation observed between levels of antibodies to the X and Y parasite preparations is consistent with a limited antigenic heterogeneity of *T. vaginalis* (3).

Of particular interest appears to be our observation that antibody levels detected by the ELISA were related to a previous or current local detection of the parasite. ELISA-detected antibodies were present in 85% of patients in whom the current presence of the parasite was demonstrated, a figure higher than that previously reported with a similar method (25). The clinical significance of antibodies detected by ELISA is also evidenced by the presence of antibodies in 64% of patients with proven previous *T. vaginalis* infection but in whom no parasite could currently be found. These patients often had a history of previous venereal disease. In addition, ELISA has several advantages such as its practicability and better definition of antigens, especially for soluble preparations. The respective development of a local or serum antibody response to local bacterial, parasitic, or viral antigens may be controlled by several influences, such as the nature of the antigen or pathogen, its live or inactivated form, its local amount, and the frequency and length of stimulation. The correlation between the presence of a serum antibody response to *T. vaginalis* and active infection was better than that between infection and local antibodies. In our study, circulating IgA antibodies were detected in

TABLE 1. Relation between ELISA and immunofluorescent antibodies detected in sera, previous record of trichomonasis or other sexually transmitted diseases, and presence or absence of *T. vaginalis* in direct examination or culture, or both

Direct examination[a] or culture	Previous record of trichomoniasis	No. of antibodies detected in sera			
		E+, I+[b]	E+, I−	E−, I+	E−, I−
+	0	20	4	0	1
	+	5	4	1	4
−	0	19 (7)[c]	10 (5)	3 (1)	27 (1)

[a] *T. vaginalis* detected (+) or not detected (−).
[b] E+, ELISA positive; E−, ELISA negative; I+, immunofluorescence positive; I−, immunofluorescence negative.
[c] Number of patients with previous sexually transmitted diseases.

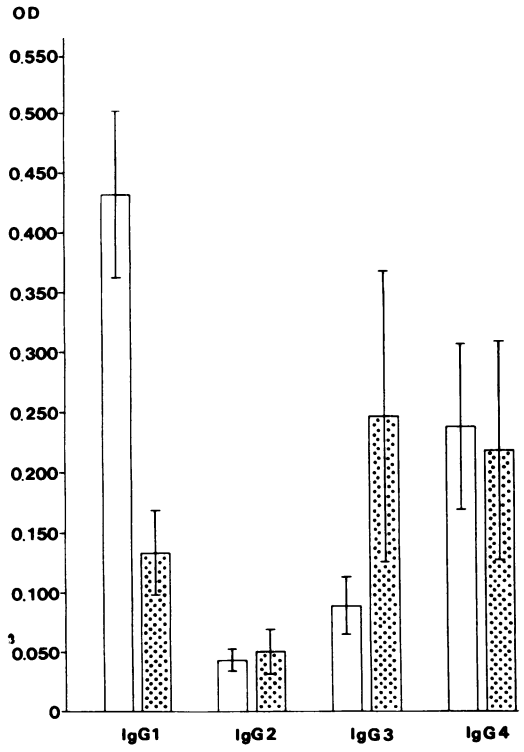

FIG. 6. Distribution of the levels of serum antitrichomonal antibodies of the IgG1, IgG2, IgG3, and IgG4 subclasses in seven male (□) and 19 female (▨) patients. Values were obtained in ELISA and expressed as OD_{405} values corresponding to a 1:200 serum dilution.

infected patients and found to be related to their clinical status, but less significantly than were total antitrichomonal antibodies. The presence of IgG antibodies in serum may be related to a local response of IgG-secreting cells which was previously reported (2, 26) but may also represent a systemic response to released antigen. The finding of antibodies of the IgG4 subclass, comparatively elevated in some patients, is consistent with repeated antigenic stimulations (1). Although few infected male patients were studied, it is noteworthy that they differed from infected female patients in their comparatively low level of antibodies of the IgG1 subclass. This difference may depend on either sex or the method of immunization. Sera from infected patients have been shown to exhibit a complement-mediated lytic activity on trichomonads in cultures (13) and to exert protection against experimental trichomoniasis in rodents (27). In human trichomoniasis, additional information is needed to evaluate the protective role of antibodies of the IgG class.

LITERATURE CITED

1. **Aalberse, R. C., R. van der Gaag, and J. Leeuwen.** 1984. Serological aspects of IgG4 antibodies: prolonged immunization results in an IgG4-restricted response. J. Immunol. **130**:722–726.
2. **Ackers, J. P., W. R. Lumdsen, R. D. Catterall, and R. Coyle.** 1975. Antitrichomonal antibody in the vaginal secretions of women infected with *Trichomonas vaginalis*. Br. J. Vener. Dis. **51**:319–323.
3. **Alderete, J. F.** 1983. Antigen analysis of several pathogenic strains of *Trichomonas vaginalis*. Infect. Immun. **39**:1041–1047.
4. **Aure, J. C., and H. Gjonnaess.** 1969. Metronidazole treatment of Trichomonal vaginitis. Acta Obstet. Gynecol. Scand. **48**: 440–445.
5. **Brasseur, P., A. Menard, and Y. Michel.** 1984. Interest of indirect immunofluorescence and indirect haemagglutination tests for the detection of urogenital trichomoniasis. Ann. Biol. Clin. **42**:343–348.
6. **Brasseur, P., and J. Savel.** 1982. Cytopathogenic effect of different strains of *Trichomonas vaginalis* in cell culture and quantitative evaluation of virulence. C. R. Soc. Biol. **176**: 849–860.
7. **Diamond, L. S.** 1957. The establishment of various trichomonads of animals and men in axenic cultures. J. Parasitol. **43**:488–490.
8. **Diddle, A. W.** 1967. *Trichomonas vaginalis*: resistance to metronidazole. Am. J. Obstet. Gynecol. **98**:583–585.
9. **Felman, Y. M., and J. A. Nikitas.** 1979. Trichomoniasis, candidiasis, and Corynebacterium vaginale vaginitis. N.Y. Med. J. **79**:1563–1566.
10. **Fouts, A. C., and S. J. Kraus.** 1980. *Trichomonas vaginalis*: reevaluation of its clinical presentation and laboratory diagnosis. J. Infect. Dis. **141**:137–143.
11. **Fuller, A. T.** 1938. The formamide method for the extraction of polysaccharides from haemolytic streptococci. Br. J. Exp. Pathol. **19**:130–139.
12. **Hoffmann, B.** 1966. An evaluation of the use of the indirect haemagglutination method in the serodiagnosis of trichomonadosis. Wiad. Parazytol. **12**:392–397.
13. **Holbrook, T. W., R. J. Boackle, J. Vesely, and B. W. Parker.** 1982. *Trichomonas vaginalis*: alternative pathway activation of complement. Trans. R. Soc. Trop. Med. Hyg. **76**:473–475.
14. **Jaakmees, H., J. Teras, E. Roigas, U. Nigesen, and H. Tompel.** 1966. Complement-fixing antibodies in the blood sera of men infested with *Trichomonas vaginalis*. Wiad. Parazytol. **12**: 378–383.
15. **Kramar, J., and K. Kucera.** 1966. Immunofluorescence demonstration of antibodies in urogenital trichomoniasis. J. Hyg. Epidemiol. Microbiol. Immunol. **10**:85–88.
16. **Kuberski, T.** 1978. Evaluation of the indirect haemagglutination technique for study of *Trichomonas vaginalis* infections, particularly in men. Sex. Transm. Dis. **5**:97–102.
17. **Mason, P. R.** 1979. Serodiagnosis of *Trichomonas vaginalis* infection by the indirect fluorescent antibody test. J. Clin. Pathol. **32**:1211–1215.
18. **McEntegard, M. G.** 1952. The application of haemagglutination technique to the study of *Trichomonas vaginalis* infections. J. Clin. Pathol. **5**:275–280.
19. **Meingassner, J. G., and J. Thurner.** 1979. Strain of *Trichomonas vaginalis* resistant to metronidazole and other 5-nitroimidazoles. Antimicrob. Agents Chemother. **15**:254–257.
20. **Muller, M., J. G. Meingassner, W. A. Miller, and W. J. Ledger.** 1980. Three metronidazole resistant strains of *Trichomonas vaginalis* from the United States. Am. J. Obstet. Gynecol. **138**:808–812.
21. **Peterson, K. M., and J. F. Alderete.** 1982. Host plasma proteins on the surface of pathogenic *Trichomonas vaginalis*. Infect. Immun. **37**:755–762.
22. **Peterson, K. M., and J. F. Alderete.** 1983. Acquisition of α_1-antitrypsin by a pathogenic strain of *Trichomonas vaginalis*. Infect. Immun. **40**:640–646.
23. **Reardon, L. V., L. L. Ashburn, and L. Jacobs.** 1961. Differences in strains of *Trichomonas vaginalis* as revealed by intraperitoneal injections into mice. J. Parasitol. **47**:527–532.
24. **Robinson, S. C.** 1962. Trichomonal vaginitis resistant to metronidazole. Can. Med. Assoc. J. **86**:665–671.
25. **Street, D. A., D. Taylor-Robinson, J. P. Ackers, N. F. Hanna, and A. McMillan.** 1982. Evaluation of an enzyme-linked immunosorbent assay for the detection of antibody to *Trichomonas vaginalis* in sera and vaginal secretions. Br. J. Vener. Dis. **58**:330–333.
26. **Su, K. E.** 1982. Antibody to *Trichomonas vaginalis* in human cervicovaginal secretions. Infect. Immun. **37**:852–857.
27. **Teras, J.** 1963. On the immunogenic properties of *Trichomonas vaginalis*, p. 33–42. *In* J. Teras (ed.), Genitourinary trichomoniasis. Estonian S.S.R. Academy of Science, Tallinn, U.S.S.R.
28. **Teras, J., U. Nigesen, H. Jaakmees, E. Roigas, and H. Tompel.** 1966. The agglutinogenic properties of *T. vaginalis* in human organisms. Wiad. Parazytol. **12**:370–377.
29. **Torian, B. E., R. J. Connelly, R. S. Stephens, and H. H. Stibbs.** 1984. Specific and common antigens of *Trichomonas vaginalis* detected by monoclonal antibodies. Infect. Immun. **43**:270–275.
30. **Valent, M., R. Holkova, G. Catar, J. Klepanek, and A. Janoska.** 1976. Possibilities of serologic diagnosis of trichomoniasis by means of indirect immunofluorescence reaction. Bratisl. Lek. Listy **66**:87–92.
31. **Zil'Man, S. L.** 1978. Accelerated IFR Abs. method with blood for the serodiagnosis of urogenital trichomoniasis. Vestn. Dermatol. Venerol. **5**:26–31.