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Phenotypic Variation and Diversity among *Trichomonas vaginalis* Isolates and Correlation of Phenotype with Trichomonal Virulence Determinants

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The extent and nature of heterogeneity among representative *Trichomonas vaginalis* isolates were evaluated by flow cytofluorometry analysis. Monoclonal antibody and monospecific antiserum to an immunodominant trichomonad surface glycoprotein with a molecular weight of 267,000 (267K glycoprotein) were used to evaluate fresh isolates (JHH and RU375) and long-term grown isolates (NYH286, IR78, and JH31A) of *T. vaginalis*. Isolates NYH286, JH31A, and JHH were made up of heterogeneous staining (positive [pos] phenotype) and nonstaining (negative [neg] phenotype) populations of trichomonads, whereas RU375 and IR78 were all neg phenotype parasites. Flow cytofluorometric patterns of agar clones derived from single organisms of heterogeneous isolates such as NYH286 showed populations which were either homogeneous pos or neg and also showed clones which were heterogeneous in nature containing both phenotypes. Fluorescence-activated cell sorting was also accomplished, and subpopulations of defined pos or neg phenotype were purified. Flow cytofluorometry evaluation of all isolates for an extended period revealed a phenotypic variation among all heterogeneous isolates and also for all clones and subpopulations derived from the heterogeneous isolates. On the other hand, IR78 and RU375 did not undergo phenotypic variation and have remained neg for greater than 4 years. Parasites which were nonreactive with either monoclonal antibody or monospecific antiserum to the 267K glycoprotein in flow cytofluorometry did not possess the antigen on their surface. This was determined by radioimmunoprecipitation assays using extracts of iodinated trichomonads. Finally, neg phenotype parasites yielded enhanced rates of contact-dependent cytotoxicity of host cell monolayers as compared with the pos phenotype trichomonads.

Work by us and others performing antigenic analysis among *Trichomonas vaginalis* isolates has shown the complex nature of the immunochemical composition of the pathogenic human trichomonads (1, 2, 7, 9, 13, 15, 17, 18, 20–24, 26, 27). Although an extensive commonality among *T. vaginalis* isolates, based on one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (2, 7, 13, 18, 24) and combined isoelectric focusing-SDS-PAGE (7), has been demonstrated by numerous laboratories, other groups have reported on the existence of trichomonal isolates with different antigenic compositions (15, 21–24, 26, 27). It has recently been suggested that much of the conflict regarding the antigenic and surface distinctions of *T. vaginalis* is due to the presence on or absence from trichomonal surfaces of a repertoire of high-molecular-weight proteins (10). Furthermore, trichomonads without the surface markers still synthesize the proteins within the important repertoire responsible for antigenic heterogeneity (10).

More recent experiments, which used a monoclonal antibody (MAb) reactive to a high-molecular-weight glycoprotein present within the important group of immunogens, also showed a differentiation of *T. vaginalis* isolates and subpopulations of parasites of certain isolates (9). Consistent with earlier findings (10), organisms without the surface glycoprotein (negative [neg] phenotype) still synthesized the molecule, albeit at diminished levels (9). It was noteworthy that isolates lacking the surface immunogens failed to elicit high-titered antibody to these proteinaceous molecules during experimental infections, thus explaining some of the varied reactions obtained previously by others who used conventional immunologic assays with antiserum reagents (15, 21–23). These initial data support the hypothesis that the surface disposition of key immunogens is partially responsible for the reported extensive antigenic diversity among *T. vaginalis* (7, 9, 10).

It became necessary, therefore, to study the extent of heterogeneity of *T. vaginalis* based on specific surface components such as the glycoprotein immunogen recently characterized (9). Also, we wanted to test whether the pathogenic human trichomonads underwent phenotypic variation and the possible role, if any, of this property with established virulence traits for this protozoan (5, 6, 8–10, 15). In this study, we showed that *T. vaginalis* was indeed subject to phenotypic variation with respect to a specific, highly immunogenic glycoprotein (9). Trichomonal isolates and clones were either heterogeneous populations having trichomonads with and without the surface glycoprotein or were homogeneous for the absence of this membrane marker. The data also indicated that a relationship may exist between the phenotype of trichomonads and their ability to efficiently elicit contact-dependent host cytopathogenicity (6, 8). These results appear very meaningful in terms of clarifying past discrepancies regarding the reported extensive antigenic heterogeneity and virulence properties of the pathogenic human trichomonads (15, 16, 21–24, 26, 27). We discuss the biological implications of our results.

MATERIALS AND METHODS

Growth and radiolabeling of organisms. All of the *T. vaginalis* isolates used here have been used previously (2, 6, 7, 9, 10, 19) and were grown in trypticase-yeast extract-maltose (TYM) medium (12) supplemented with 10% heat-

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inactivated horse serum (2, 9). Isolates NYH286, JH31A, and IR78 were passaged daily for 2 years, and isolates RU375 and JHH were grown for less than 1 week before initial evaluation with MAb. Only mid- to late-logarithmic-phase organisms (18) were used in experiments. *Trichomonas tenax*, a nonpathogenic human trichomonad, was obtained from American Type Culture Collection, Rockville, Md., and was passaged in Trypticase-Panmede (Harcos, Inc., Bronxville, N.Y.) medium with 10% serum (6, 8, 19). Intrinsic and extrinsic radiolabeling of trichomonads used for radioimmunoprecipitation (RIP) experiments were performed as described recently (2, 3, 18). Details for cytadherence experiments using [^{3}H]thymidine-labeled trichomonads incubated with HeLa cells in monolayer culture were also reported recently (6).

Cloning of *T. vaginalis*. Agar cloning of isolates NYH286, JH31A, and JHH trichomonads was performed by using a modification of a published procedure (14). Briefly, 35.5 ml of freshly prepared TYM medium containing 0.65% ionagar (Difco Laboratories, Detroit, Mich.) was autoclaved and then incubated in a 60°C water bath. The medium was then transferred to a 37°C water bath, and 0.4 ml of stock penicillin and streptomycin solutions (100 U/ml and 100 μg/ml final concentrations, respectively), 4 ml of serum, and 0.1 ml of inoculum containing 10 to 100 organisms were added immediately upon temperature equilibration. The contents were mixed before addition to sterile glass petri dishes (100 by 15 mm) with Whatman filter paper taped to the inside of the glass cover. The plates were placed in a Brewer jar with GasPak (BBL Microbiology Systems, Cockeysville, Md.) at 37°C and monitored daily. Colonies were visible by 3 days and were picked with sterile glass Pasteur pipettes for inoculation into TYM-serum medium. Clones were cryopreserved in liquid nitrogen, and the time from in vitro cultivation of clones to cytofluorometric analysis never exceeded 2 days unless otherwise stated.

Flow cytofluorometry and fluorescence-activated cell sorting (FACS). Preparation of trichomonads for indirect immunofluorescence was similar to the procedure recently described (3, 10). Briefly, 2×10^6 organisms were washed twice in phosphate-buffered saline PBS (2, 18) before suspending the pellet in hybridoma supernatant containing immunoglobulin G2A (IgG2a) MAb designated C20A3. This MAb has been recently characterized (9) and reacts with a high-molecular-weight (267,000) glycoprotein (267K glycoprotein) of *T. vaginalis* isolate NYH286. Hybridoma supernatant with MAb of the same isotype but to *Mycoplasma pneumoniae* was kindly provided by Joel Baseman of this department and was used as a control. After incubation for 20 min at 4°C, the parasites were washed three times in cold PBS. The organisms were then incubated for 20 min at 4°C in PBS with a 1:20 final dilution of the IgG fraction of fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Cooper Biomedical, Inc., West Chester, Pa.). After washing the organisms three additional times, flow cytofluorometric analysis was performed by using a Becton Dickinson FACS-IV instrument preequilibrated with known fluorescence probes. Data are presented on the basis of log fluorescence intensity versus parasite number. Different concentrations of MAb IgG purified by protein A-Sepharose chromatography (Pharmacia Fine Chemicals, Piscataway, N.J.) were also tested. Monospecific antiserum to the trichomonad glycoprotein prepared as described below was also used, and results were identical to those obtained with C20A3. All analysis involving *T. vaginalis* isolates, clones, and purified subpopulations were run in triplicate for each experimental condition examined and were repeated no less than three separate times. Trichomonad populations which changed in phenotype at certain time points as described in the text were also cryopreserved and reassayed at later times for phenotypic variation. Results were identical to those obtained for the continuously passaged cultures of the same isolates.

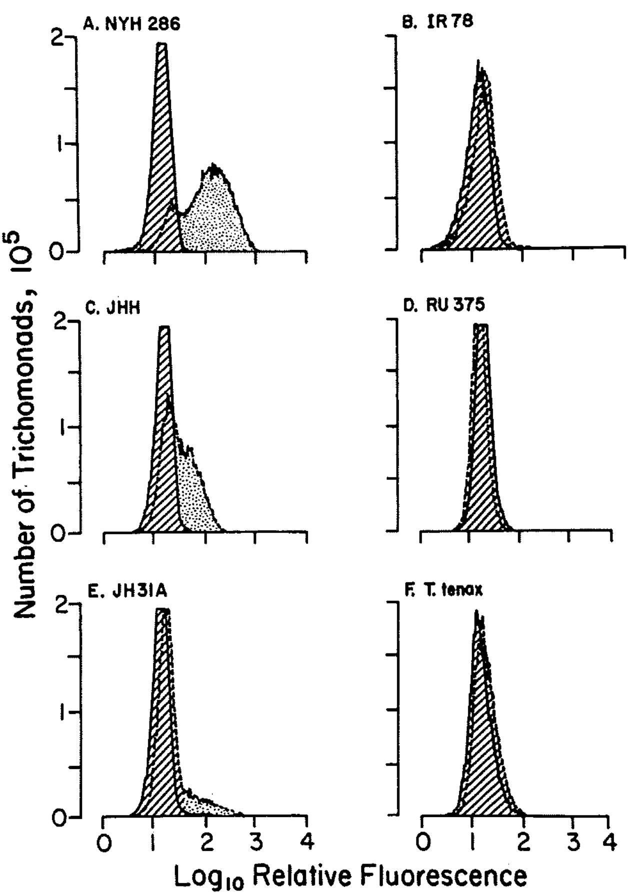


FIG. 1. Flow cytofluorometry analysis with C20A3 (- - -) and irrelevant (——) MAb of representative long-term grown (A, B, and E) versus fresh (C and D) isolates of *T. vaginalis* and a nonpathogenic trichomonad, *T. tenax* (F). Parasites were treated with hybridoma supernatant containing MAb C20A3 or irrelevant antibody and remained highly motile throughout the assay. [hatched], Neg phenotype (lack of fluoresence); [stippled], pos phenotype.

Before sorting trichomonads on the basis of presence or absence of fluorescence (positive [pos] and neg phenotype, respectively), the sample tubing of the FACS-IV instrument was flushed with detergent, 95% ethanol, and sterile distilled water. The tubing was then rinsed for 30 min each with 10 ml of gentamicin solution (50 mg/ml) followed by 10 ml of 100 U of penicillin–100 μg of streptomycin solution per ml. During FACS analysis, the parasites were collected in sterile plastic tubes (10 by 75 mm) containing 1 ml of TYM medium with antibiotics. The medium was then transferred to standard culture tubes containing additional medium, and after achieving appropriate cell densities (18), the subpopulations were reevaluated by flow cytofluorometry.

Contact-dependent HeLa cell killing. The quantitative, colorimetric assay to measure cytadherence-mediated cytotoxicity of HeLa cells in monolayer culture was performed as described recently (6, 8).

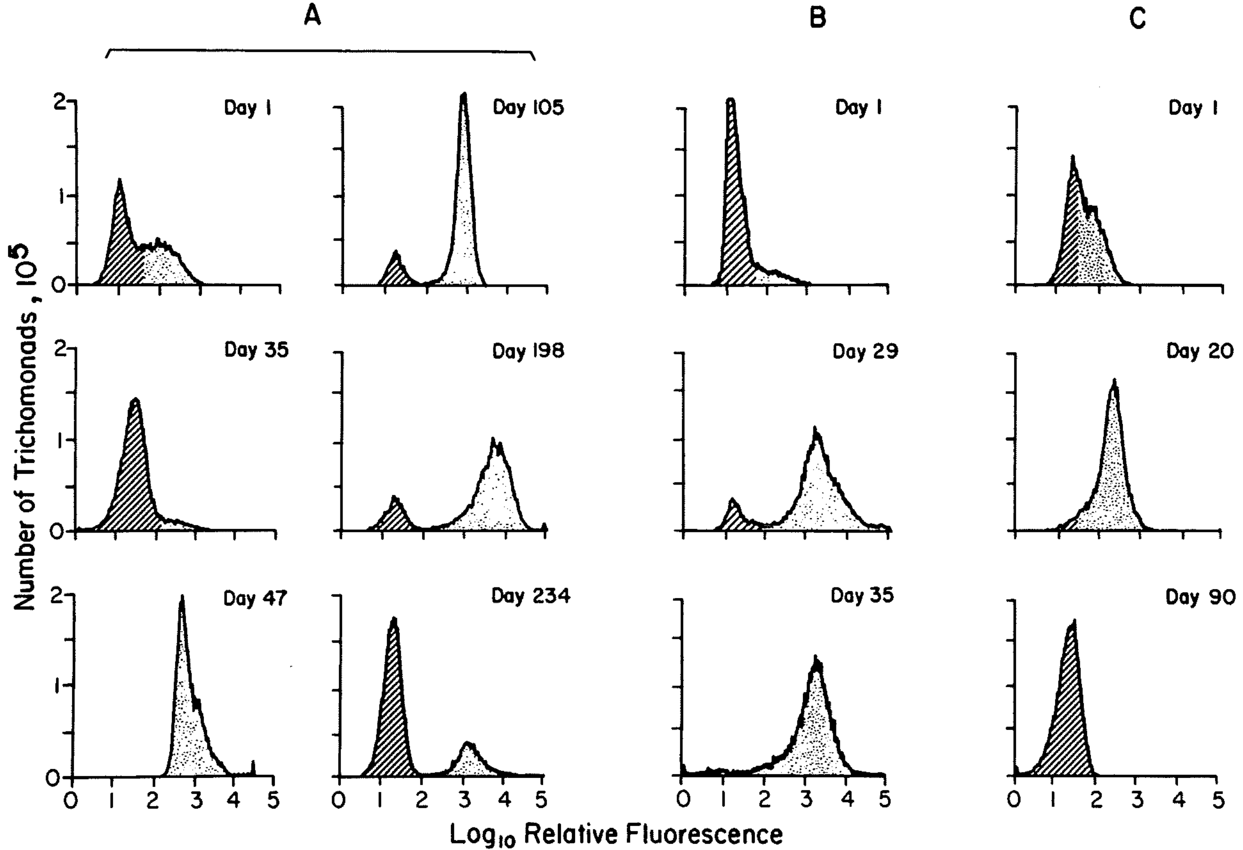


FIG. 2. Phenotypic variation determined by flow cytofluorometry with C20A3 MAb among trichomonads in the parent population of heterogeneous *T. vaginalis* NYH286 (A), JH31A (B), and JHH (C) isolates. Days were selected which represented overall dramatic shifts from the previous timepoint in the ratio of respective neg and pos phenotypes. ▨, Neg phenotype; ▭, pos phenotype trichomonads.

RIP assay and SDS-PAGE. Procedures detailing the RIP assay and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were as reported elsewhere (2, 3, 18).

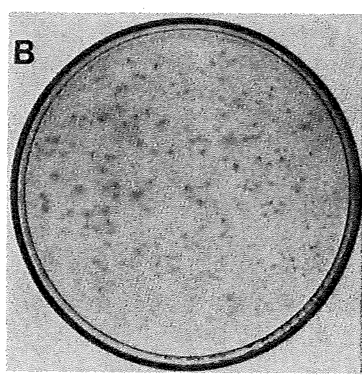
Purification of trichomonad antigen and generation of monospecific antiserum. Because of results which show that lack of fluorescence of trichomonads with the MAb could be due to epitope inaccessibility and not to absence of antigen from parasite surfaces, it was necessary to generate a monospecific antiserum to the important glycoprotein. Purified IgG2a MAb from ascites fluid or hybridoma supernatants was coupled to CNBr-activated Sepharose 4B (Sigma Chemical Co., St. Louis, Mo.) (11). Approximately 10^9 sorted pos phenotype parasites of isolate NYH286 (see Fig. 5) were solubilized with Zwittergent 3-12 (Z3-12) detergent (Calbiochem-Behring, La Jolla, Calif.) as performed for RIP (2, 3). The detergent extract was then subjected to immunoaffinity chromatography at 4°C at a rate of 5 ml/cm^2 per h. After extensive washing of the column with at least 10 volumes of PBS, trichomonad antigen was eluted with 3 M NaSCN (pH 7.0). The adherent material was then dialyzed against two changes of 4 liters of distilled, deionized H_2O before concentrating by ultrafiltration by using an XM100A membrane (Amicon Corp., Lexington, Mass.). Purity of the trichomonad antigen was confirmed by immunoblot (9, 10, 25) with MAb and monospecific antiserum to the 267K glycoprotein and also by using rabbit antiserum to total *T. vaginalis* NYH286 proteins (2, 3).

New Zealand White male rabbits were then immunized by subcutaneous and intramuscular injection of 0.2 mg of antigen in Freund complete adjuvant. Rabbits were then given three additional booster injections using the same amount of antigen in Freund incomplete adjuvant. Antibody synthesis was monitored by enzyme-linked immunosorbent assay (4), and serum specificity was tested by immunoblot using total trichloroacetic acid-precipitated *T. vaginalis* proteins (2, 9).

RESULTS

Flow cytofluorometric analysis and phenotypic variation of *T. vaginalis* isolates and clones. Medium without MAb to the glycoprotein immunogen or an MAb of the same isotype but nonreactive with *T. vaginalis* based on various criteria (9) was used as a negative control to establish base-line fluorescence for trichomonads. These controls also demonstrated no detectable staining of parasites based on indirect immunofluorescence or other assays (9). Thus, the distribution of organisms with irrelevant MAb established arbitrary units for control or negative phenotype organisms (Fig. 1).

Figure 1 shows the flow cytofluorometric patterns of representative *T. vaginalis* long-term grown (NYH286, JH31A, and IR78) and fresh (JHH and RU375) isolates when the C20A3 MAb reactive with a trichomonad immunogen was used. Only two types of parent populations were obtained, based on fluorescence levels above control values. Isolates NYH286, JHH, and JH31A were heterogeneous, and isolates IR78 and RU375 were representative of homogeneous, neg phenotype trichomonads. *T. tenax*, a nonpath-



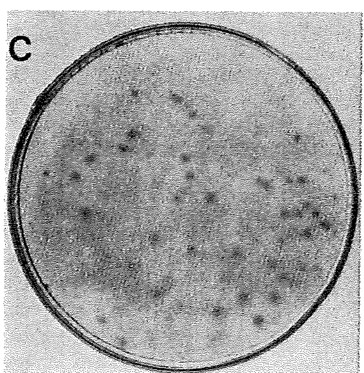


FIG. 3. Representative pictures showing agar growth of individual colonies for heterogenous NYH286 (A) as well as clone 1 (B) and clone PH4 (C) of NYH286. The heterogeneous distribution of NYH286 at the time of agar cloning is shown in Fig. 1.

ogenic trichomonad, also was nonreactive with C20A3 MAb (Fig. 1F) and does not appear to synthesize the glycoprotein antigen (9). Ten additional isolates were evaluated by cytofluorometry with the MAb, and the phenotypes were the same as presented above for the trichomonal isolates used in this study. Finally, cytofluorometric analysis of duplicate cultures of all isolates using monospecific antiserum to the trichomonad glycoprotein gave identical results (Fig. 1). These data demonstrate that the pos and neg phenotype designations are due solely to the presence or absence of the antigen on *T. vaginalis* surfaces, respectively.

We next monitored the three heterogeneous isolates (Fig. 1A, C, and E) over an extended time frame. This was necessary because daily analysis of the parent populations showed no significant phenotypic changes. Figure 2 shows the dramatic shifts in the number of trichomonads expressing either pos or neg phenotype which occurred during prolonged in vitro growth. These data illustrate the dynamic nature in *T. vaginalis* populations for the expression of a specific surface immunogen. Isolates IR78 and RU375 failed to produce any phenotypic changes even after 2 years of in vitro cultivation, and interestingly, no stable homogeneous pos phenotype populations have been observed among trichomonal isolates.

Phenotypic differences of agar clones of *T. vaginalis*. We then obtained agar clones of a heterogeneous population of NYH286. Colonies of the parent population and of two representative clones, clone 1 and clone PH4, are shown in Fig. 3. Clone 1 (Fig. 3B) was light-colored and always gave small, discrete colonies, and clone PH4 (Fig. 3C) produced more diffuse, darker colonies.

Flow cytofluorometric analysis of numerous clones of NYH286 is presented in Fig. 4. As expected, certain clones were comprised of homogeneous pos (clones PH3 and PH4) or neg (clone 3) phenotype populations. Other clones (1, 9, and 10) were heterogeneous populations consisting of trichomonads with and without the specific surface glycoprotein. As expected, no fluorescence was detected with control supernatant or irrelevant MAb.

Monitoring of all clones over several weeks demonstrated phenotypic changes among the trichomonad population, as was seen for representative isolates (Fig. 2). All of 50 clones evaluated, for example, demonstrated pos-to-neg and neg-

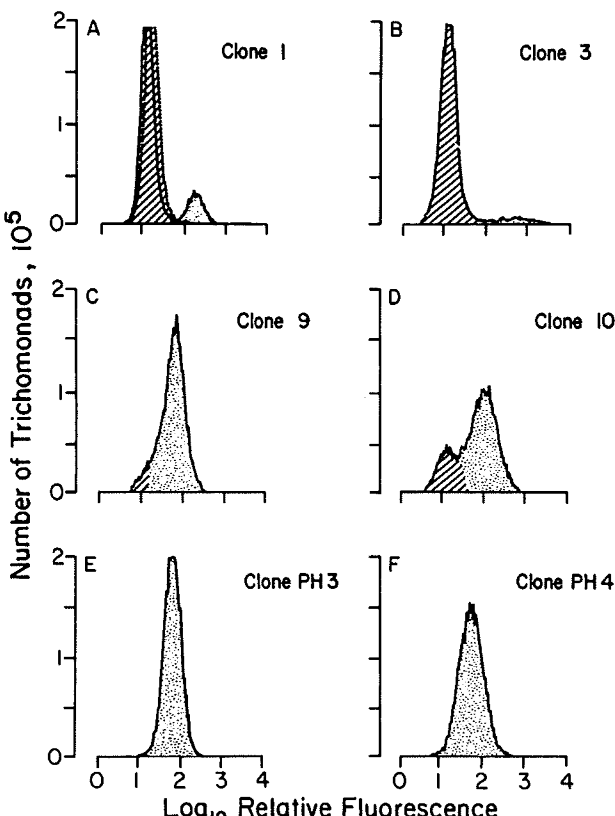


FIG. 4. Patterns from flow cytofluorometry using C20A3 MAb (▒) of six representative clones of *T. vaginalis* NYH286 obtained from the agar plate shown in Fig. 3. Note that homogeneous pos (E and F) and neg (B) populations of trichomonads in additio.. to clones which were heterogeneous (A, C, and D) from C20A3 reactivity were obtained. Lack of fluorescence using irrelevant MAb of the same isotype is shown for clone 1 (▨).

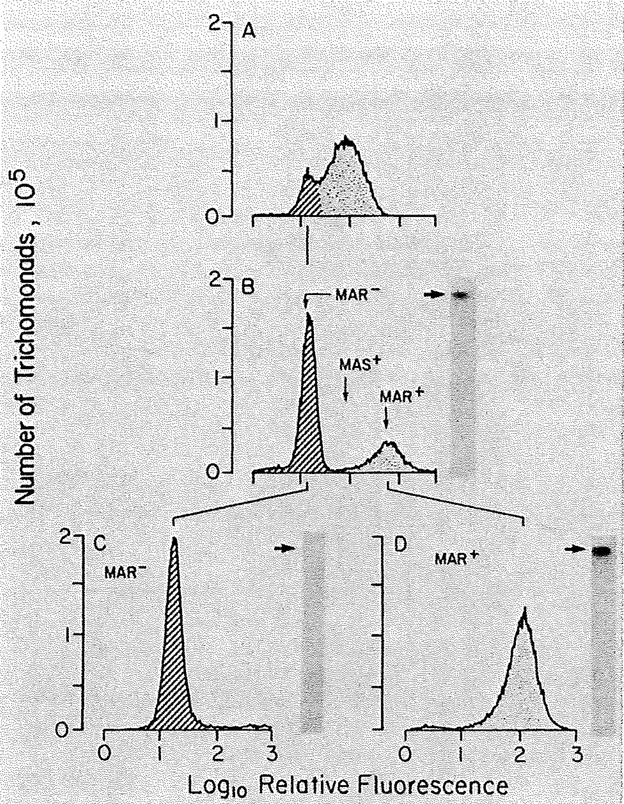


FIG. 5. FACS with C20A3 MAb of heterogeneous *T. vaginalis* NYH286 (A) into distinct subpopulations (C and D). MAR^- MAR^+, and MAS designations refer to resistance and susceptibility to MAb C20A3-mediated lysis of trichomonads, and plus (+) and minus (−) refer to fluorescence. The gel lanes to the right of FACS patterns show the autoradiograms after RIP and SDS-PAGE of immunoprecipitated surface glycoprotein with MAb (5). Note that only homogeneous pos or heterogeneous populations of trichomonads readily yield a precipitable high-molecular-weight iodinated glycoprotein. ▭, Neg phenotype; ▭, pos phenotype.

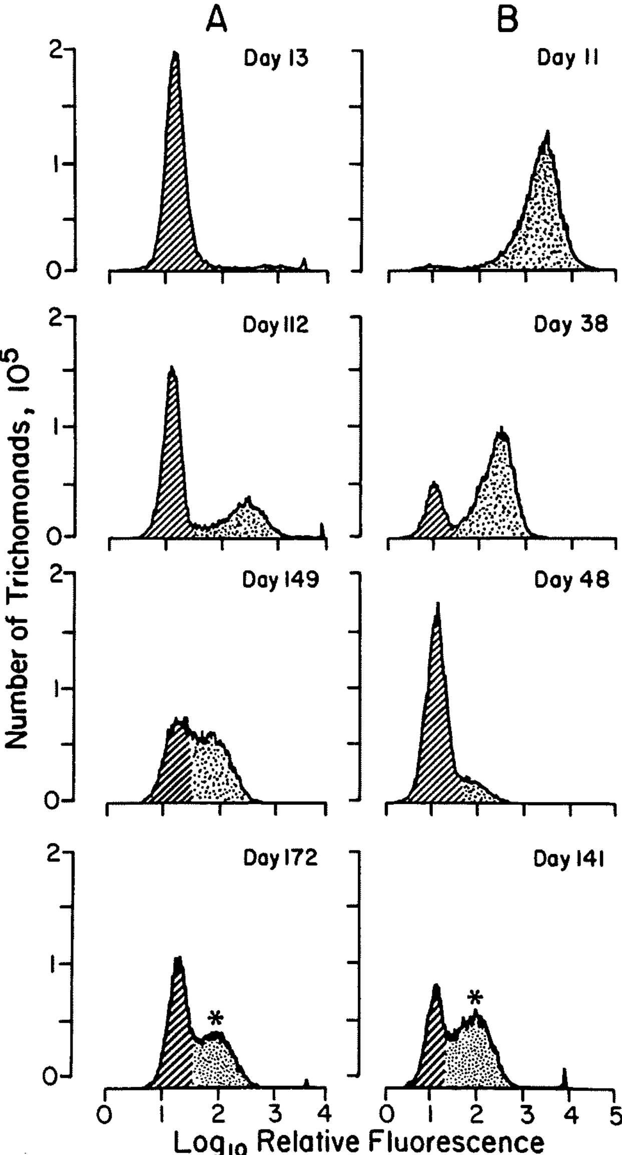


FIG. 6. Phenotypic variation of sorted MAR^- and MAR^+ (Fig. 5) subpopulations of *T. vaginalis* isolate NYH286. *, Pos phenotype trichomonads with fluorescence intensity less than MAR^+, which were readily lysed with high concentrations of C20A3 IgG as described in the text. ▨, Neg phenotype; ▭, pos phenotype.

to-pos shifts in reactivity to C20A3 MAb. All pos phenotype clones became either neg or heterogeneous for the specific surface marker after several weeks; no stable pos phenotype trichomonads were obtained. Finally, similar results were observed for clones of other heterogeneous isolates such as JH31A and JHH (Fig. 1).

FACS of *T. vaginalis* subpopulations. In addition to studying cloned parasites, we attempted to obtain subpopulations of *T. vaginalis* from isolates such as NYH286 or JH31A. Incubation of these isolates for several days with high levels (100 μg/ml) of C20A3 IgG resulted in lysis of most but not all organisms expressing the surface glycoprotein (Fig. 5B). Interestingly, a subpopulation of resistant trichomonads with a higher fluorescence intensity was obtained. In this way, three subpopulations were ultimately delineated with the C20A3 MAb and were designated MAb-resistant, neg phenotype (MAR^-), MAb-resistant, pos phenotype (MAR^+), and MAb-sensitive, pos phenotype (MAS^+) trichomonads.

Respective phenotypes were fractionated by FACS as shown in Fig. 5C and D. An RIP assay (9) with C20A3 MAb and a detergent extract of either iodinated MAR^- or MAR^+ subpopulations showed that only MAR^+ parasites possessed surface glycoprotein. Monospecific antiserum to the purified trichomonad glycoprotein yielded identical results, confirming that neg phenotype parasites were indeed without the surface antigen.

Phenotypic variation of sorted MAR^- and MAR^+ parasites of isolates NYH286 and JH31A is presented in Fig. 6 and 7, respectively. Again, the generation of a subpopulation with the opposite phenotype was observed after in vitro growth for extended periods. Interestingly, pos phenotype organisms for both NYH286 and JH31A originally resistant to 100 μg of C20A3 IgG per ml gave rise to trichomonads susceptible to MAb-mediated lysis, thus comprising mostly MAS^+ phenotype organisms (Fig. 5). Only upon reexposure to antibody for several days could MAR^+ parasites with increased fluorescence intensity again be generated.

Differential rates of host cytotoxicity were related to pheno-

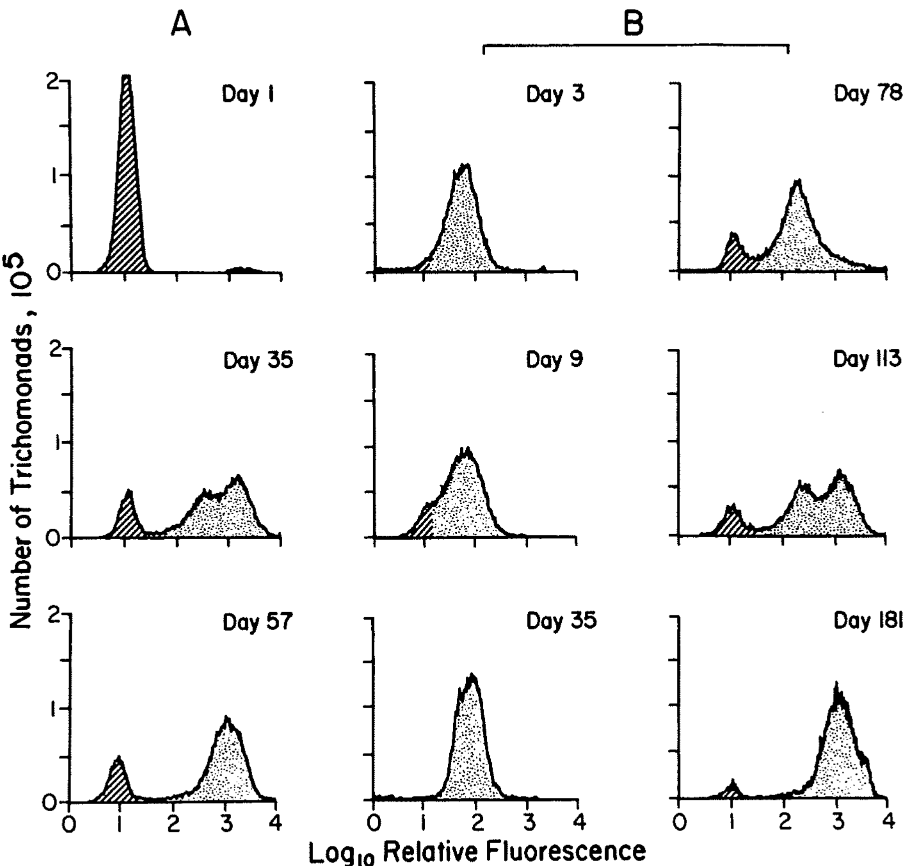


FIG. 7. Phenotypic variation for C20A3 monoclonal antibody-sorted MAR^- (A) and MAR^+ (B) subpopulations of *T. vaginalis* isolate JH31A. Note that unlike isolate NYH286, in which MAR^+ dramatically shifted to MAR^- phenotype (Fig. 6), the JH31A MAR^+ (B) organisms never yielded >20% of the parasites of neg phenotype.

type. We recently developed a colorimetric, quantitative assay to demonstrate cytadherence-mediated host cytotoxicity (6, 8). Cells in monolayer culture were readily killed after coincubation with *T. vaginalis* for 16 to 18 h. We therefore wanted to test the ability of pos and neg phenotypes of *T. vaginalis* to elicit contact-dependent host cell death. Figure 8 shows results from a representative experiment which demonstrates a typical HeLa cell monolayer before and after (Fig. 8A1 and Fig. 8A2 through A4, respectively) incubation with *T. vaginalis* NYH286. As can be seen, only populations with neg phenotype trichomonads (Fig. 8B3 and B4) totally disrupted the cell monolayers.

Additional data showing the extent of host cell killing by representative clones and subpopulations are presented in Table 1. As described above, all pos phenotype clones and subpopulations (MAR^+) yielded diminished levels of host cytotoxicity. On the other hand, trichomonal populations with neg phenotype trichomonads were effective in host cell killing under these conditions. Finally, in all cases, a change in phenotype from pos to neg paralleled the enhanced ability of trichomonads to readily disrupt cell monolayers (Fig. 8). These experiments have been conducted more than 10 times, and a Student t test performed on results from these cytotoxicity assays (8) yielded a P value of ≤ 0.001, indicating a correlation between parasite phenotype and the rate of killing of cells in monolayer culture.

We finally tested whether longer incubations would in fact result in cell killing by the pos phenotype trichomonads. Pos phenotype clones and subpopulations of *T. vaginalis* NYH286 could indeed produce cell death after extended incubation times (Fig. 9). No change in phenotype occurred during this prolonged incubation with host cells.

DISCUSSION

The involvement of a *T. vaginalis* surface immunogen in phenotypic variation and antigenic heterogeneity was demonstrated. This study was necessary because of recent work which showed that major antigenic distinctions among trichomonal isolates and parasite subpopulations were due to surface disposition of a repertoire of highly immunogenic proteins (9, 10). Experiments using an MAb to a major, high-molecular-weight (267K) glycoprotein of *T. vaginalis* reinforced these earlier observations (7, 10). Parasites without this surface glycoprotein (neg phenotype) also synthesized the antigen, albeit at levels lower than those found among pos phenotype trichomonads (9). In this report we show that some but not all *T. vaginalis* isolates are capable of undergoing phenotypic variation for a highly immunogenic glycoprotein originally detected within the repertoire of antigens present or absent from trichomonal surfaces (10).

Overall, the data indicate that two phenotypes are present within parent populations of most *T. vaginalis* isolates studied. Although isolates like IR78 and RU375 synthesize the glycoprotein recognized by the MAb (9), they have remained homogeneous, neg phenotype organisms. Long-term in vitro cultivation of these isolates did not yield

phenotypic variation, suggesting that these parasites are unable to sequester this key glycoprotein on their surfaces. Alternatively, this glycoprotein may be actively released or shed by these organisms as was recently suggested (5).

The fact that extended in vitro cultivation over many generations was necessary to see phenotypic variation suggests that surface perturbations as described here are not coupled to single cell cycle events. Further studies using a more defined medium might provide insight into nutritional requirements or pressures, if any, which affect the pheno-

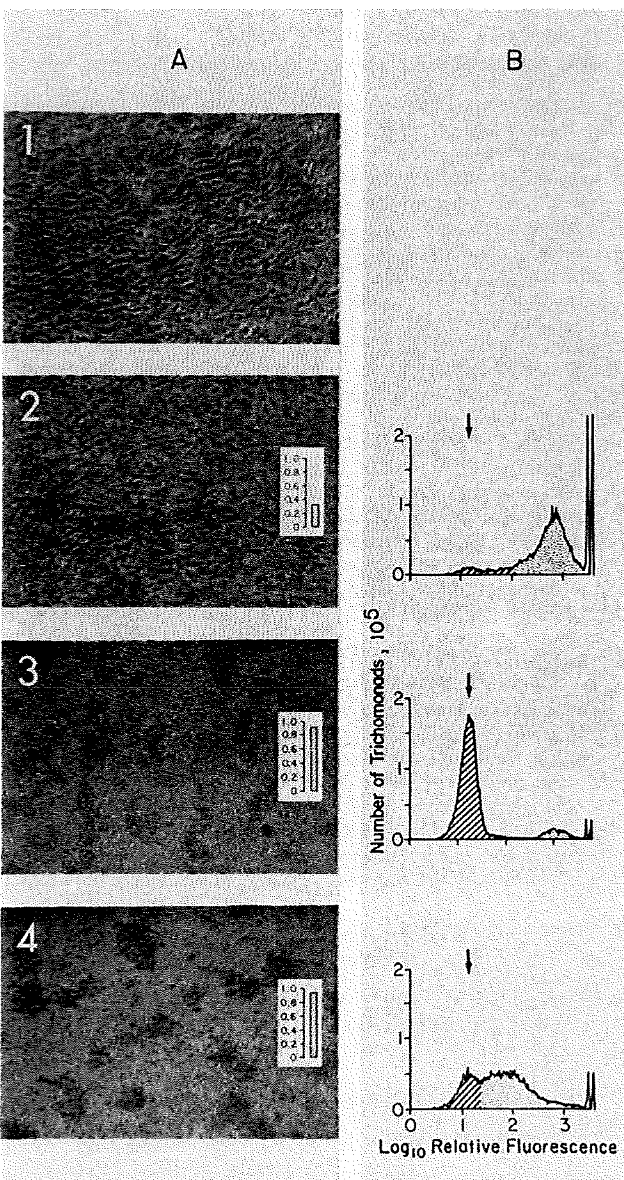


FIG. 8. Assay showing contact-dependent host cytotoxicity by *T. vaginalis* of defined phenotypes. (A) HeLa cell monolayers before (panel 1) and after (panels 2 through 4) incubation with *T. vaginalis* NYH286. (B) Corresponding flow cytofluorometric patterns using C20A3 MAb of MAR$^+$ (panel 2), MAR$^-$ (panel 3), and heterogeneous (panel 4) parasites used in the host cytotoxicity assay. Arrows, Area of neg phenotype due to absence of C20A3 reactivity. The inset in the cell monolayers (panel A) gives a measure of the extent of cell killing by the trichomonal populations as determined by the cytotoxicity assay (Table 1).

TABLE 1. Representative experiment showing the relationship between *T. vaginalis* phenotype and HeLa cell cytotoxicity

Clone or subpopulation[a]	C20A3 antibody reaction[a]	Cytotoxicity[b]	% of control
Subpopulation			
NYH286 parent	+/−	0.736 ± 0.02	100
NYH286 MAR$^-$	−	0.735 ± 0.03	100
NYH286 MAR$^+$	+	0.212 ± 0.07	33
JH31A parent	+/−	0.803 ± 0.02	100
JH31A MAR$^-$	−	0.802 ± 0.02	100
JH31A MAR$^+$	+	0.230 ± 0.05	29
Clone			
NYH286 clone 1	−	0.725 ± 0.01	99
NYH286 clone 3	−	0.742 ± 0.03	101
NYH286 clone 9	+	0.261 ± 0.02	35
NYH286 clone 10	+/−	0.730 ± 0.01	100

[a] Determination of phenotype was by flow cytofluorometry as described in Materials and Methods, and fluorescence distributions of the populations are presented in Results. +, Pos phenotype; −, neg phenotype; +/−, heterogeneous populations made up of pos and neg phenotype organisms.

[b] The colorimetric assay to measure cell killing was performed by using HeLa cell monolayers (8). After incubation of cells with trichomonads, wells were washed, and the remaining cells were fixed with formaldehyde and then stained with crystal violet. Stained material was solubilized in 1% sodium dodecyl sulfate and monitored at a wavelength of 570 nm. All measurements of experimental samples (E) were indexed to those of control samples (C) and subtracted from 1.0; i.e., cytotoxicity = 1 − E/C.

type of pathogenic human trichomonads. In addition, growth and multiplication of the parasite under more controlled conditions might allow for an understanding at the molecular level of the regulatory mechanisms involved in the modulation of expression of important and major surface immunogens of *T. vaginalis* (7, 9, 10).

It will be important to determine whether similar dynamics in a population also occur for other surface markers. For example, trichomonal isolates such as NYH286 and RU375 differ in the surface disposition of numerous high-molecular-weight antigens in addition to the high-molecular-weight glycoprotein studied here (10). Since isolation of clones and subpopulations of defined phenotype can now be performed, it should be possible to determine whether phenotypic variation (Fig. 2, 6, and 7) is coordinated for the entire repertoire of proteins earlier identified as partially responsible for antigenic heterogeneity (7, 9, 10).

Little is known about the exact mechanism or mechanisms which elicit host cell death (6, 8). Nonetheless, it is noteworthy that the rate of contact-dependent disruption of cells in monolayer cultures (6, 8) was influenced by the phenotype of the trichomonads (Fig. 8 and 9 and Table 1). The efficient killing of host cells by heterogeneous populations can more than likely be attributed to the presence of neg phenotype organisms (Fig. 8B and Table 1). Thus, since parasite attachment to host cells is a prerequisite for cell killing, the neg phenotype property appears to correspond with at least two virulence traits, namely, host cytadherence and cytotoxicity (6). Trichomonads of defined phenotype, therefore, may be exploited for possible identification of virulence factors, such as putative adhesins or toxins involved in disease pathogenesis (Fig. 8) (6).

Finally, the absence of this and possibly other surface markers within subpopulations of trichomonads would appear to ensure survival of trichomonads vulnerable to the lytic actions of antibody (Fig. 5). Alternatively, pos phenotype organisms which may be killed by host antibody (Fig. 5) might bind the existing immunoglobulins to parasite compo-

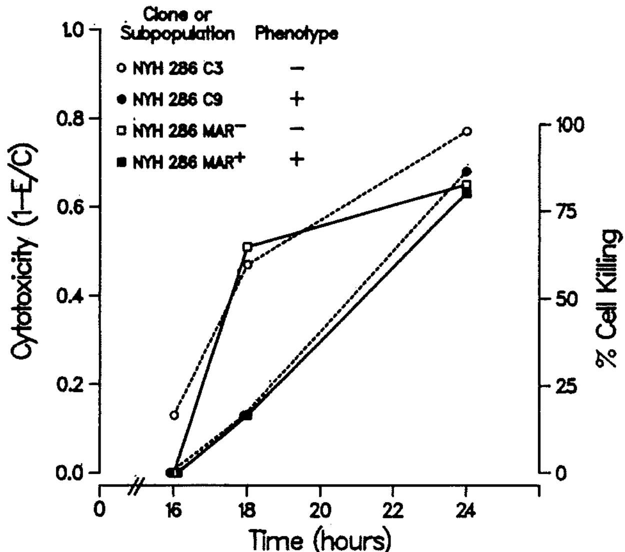


FIG. 9. Differential rates of HeLa cell killing by *T. vaginalis* NYH286 subpopulations and clones with defined C20A3 monoclonal reactions. The flow cytofluorometry patterns for respective populations of trichomonads used in the cytotoxicity assay were shown in Fig. 4 and 5, except that clone 9 was totally pos phenotype by cytofluorometry at the time of this assay. No changes in phenotype were noted throughout the duration of this experiment. The colorimetric measurement of cell monolayer disruption correlates with the percent cell killing we described recently (8).

nents also present on neg phenotype parasites. This, therefore, would provide a mechanism by which survival of some of the neg phenotype trichomonads is assured. Nonetheless, data presented in this report indicate that phenotypic variation generates neg phenotype trichomonads with enhanced virulence exhibited by their potential for immune evasion capabilities and rates of contact-dependent disruption and killing of cells in monolayer cultures (Fig. 9 and Table 1) (6, 8). Thus, phenotypic variation among *T. vaginalis* populations with respect to specific surface markers may be a significant aspect of the biology of this complex host-parasite relationship.

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