REVIEWS OF INFECTIOUS DISEASES • VOL. 10, SUPPLEMENT 2 • JULY-AUGUST 1988
 0162-0886/88/1004-0081$02.00

Alternating Phenotypic Expression of Two Classes of *Trichomonas vaginalis* Surface Markers

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The antigenic heterogeneity of *Trichomonas vaginalis* is due in part to the membrane disposition of immunogens (repertoire A) among some but not all isolates and among subpopulations of trichomonads of certain isolates. Heterogeneous *T. vaginalis* isolates undergo phenotypic variation for the A repertoire of immunogens. The presence of immunogens on (A^+ phenotype) or the absence of them from (A^- phenotype) trichomonal surfaces clearly influences the virulence traits of the pathogenic human trichomonads. A^- parasites, for example, possess an enhanced ability to cause cytadherence-dependent killing of HeLa cells as compared with A^+ parasites. A relation between phenotype of repertoire A and adherence was further substantiated by fractionating parasites of a parent *T. vaginalis* isolate, yielding adherent and nonadherent subpopulations. Trichomonad proteins were identified as putative adhesin candidates (repertoire B); adhesins were synthesized only by adherent (B^+ parasites). Flow cytofluorometry of B^+ and B^- (nonadherent) subpopulations with antibody to proteins of the A repertoire demonstrated corresponding A^- B^+ and A^+ B^- phenotypes, respectively. Data support the hypothesis that trichomonads of some isolates of *T. vaginalis* undergo alternating expression of at least two classes of surface markers.

Trichomonas vaginalis is a flagellated protozoan parasite of mucosal surfaces and is responsible for a sexually transmitted disease found throughout the world [1–4]. Reports indicate that the number of patients with trichomoniasis is high and that in the United States this microorganism causes the greatest number of illnesses due to a parasite. In all the world's societies, however, the disease imparts an emotional and economic burden.

At present, little is known about precise determinants of virulence among trichomonads or about the pathobiochemistry of the disease. Women infected with *T. vaginalis* display a broad spectrum of symptoms. Asymptomatic carriers represent a reservoir for this infectious agent, and symptomatic patients suffer severe inflammation and discomfort. Factors, of either host or parasite origin, that influence the relative resistance or susceptibility of humans to infection are also unknown. In addition no simple, rapid, and sensitive test is available for detecting the organism in individuals whose infections are not readily diagnosed by standard methods and who represent a reservoir for this parasite [5].

Finally, observations in several laboratories demonstrated dramatic heterogeneity among *T. vaginalis* isolates [6–9]. Lectins, for example, bind at different levels to various isolates [10]. Immunologic assays have shown extensive antigenic distinctions among the pathogenic human trichomonads [1, 6–9, 11, 12], especially with regard to surface components. Other biologic assays that have been unsuccessful or of limited value in correlating virulence with host symptomology (e.g., lesion size in the mouse sc assay [5, 13], hemolysis [14], and cytadherence [17]) also have demonstrated differences among *T. vaginalis* isolates. These reports have made it clear that characterization of the parasite surface was necessary to an understanding and clarification of the extent and nature of *T. vaginalis* antigenic diversity. Only then might it be possible to identify trichomonad components for the development of vaccines and immunodiagnostic tests for the control or eradication of trichomoniasis.

Data from our laboratory suggested that prominent immunogens (designated repertoire A) of *T. vaginalis* might be responsible for some aspects of the antigenic distinctions [15–21]. The surface disposi-

This work was supported by grants no. AI 18768 and AI 22380 from the National Institute of Allergy and Infectious Diseases. The author is recipient of Research Career Development Award K04 AI 00584.

The help of Diana Hinojosa in typing this manuscript is gratefully acknowledged.

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tion of immunogens of repertoire A was involved in dramatic reactions of parasite with antibody [15, 17–20]. For example, both patients' sera and monoclonal antibody distinguished two types of parent populations of *T. vaginalis*, i.e., homogeneous A^- (negative phenotype) isolates and heterogeneous isolates containing A^+ (positive phenotype) and A^- parasites. Clones or subpopulations obtained from the heterogeneous parents through fractionation experiments were found to undergo phenotypic variation. Furthermore, all of these properties were found both in isolates grown in vitro for extended periods and in fresh isolates [20]. Finally, and most importantly, virulence attributes such as host cytadherence [22, 23] and cytotoxicity [24, 25] were enhanced in A^- trichomonads [19].

These studies pointed to an important (inverse) association between *T. vaginalis* A^+ or A^- phenotype and the ability of trichomonads to adhere to cells [19, 21–23]. More specifically, alternating expression of immunogens of high molecular weight (repertoire A) and recently identified putative trichomonad adhesins (designated repertoire B) [23] appears to occur on *T. vaginalis* membranes. Evidence to support this hypothesis is reviewed.

Materials and Methods

Parasite. *T. vaginalis* NYH286 were grown in Diamond's complex medium [26] without agar that contained trypticase–yeast extract-maltose (TYM) with 10% heat-inactivated horse serum. Conditions for growth and multiplication of the organism have been described [27].

Flow cytofluorometry. Cytofluorometric analysis and fluorescence-activated cell sorting (FACS) of trichomonads were performed as described previously [17, 19].

Cytadherence and cytotoxicity. The procedures employed for measuring the ability of parasites to attach and kill HeLa cells in monolayer cultures have been described previously [22, 24].

Generation of adherent and nonadherent trichomonads. Approximately 1.5×10^7 parasites from the heterogeneous NYH286 parent isolate were incubated in 75-cm^2 flasks containing confluent monolayers of HeLa cells at 37°C for 2 hours in a CO_2 environment [22]. Adsorption of adherent organisms was performed sequentially ≤15 times until no further removal of adherent trichomonads occurred. The remaining suspension of nonadherent parasites was passaged daily and monitored for reversion to adherence capabilities.

Results

Cytadherence by A^+ and A^- trichomonal subpopulations. We have identified a group of high-molecular-weight immunogens of *T. vaginalis* [15, 16, 21] that may play a role in the reported antigenic heterogeneity of trichomonads [6–12]. This class of immunogens, designated repertoire A, undergoes phenotypic variation [19, 21]. Purified A^- subpopulations or clones of isolates without specific immunogens on their surface were found to enhance contact-dependent killing of HeLa cells [22].

It was necessary, therefore, to measure directly the extent of cytadherence among A^+ and A^- subpopulations [19]. Table 1 gives relative levels of the extent of trichomonal parasitism of HeLa cells. As can be seen, the attachment of A^- trichomonads was increased as compared with that of the A^+ counter-

Table 1. Relation between *Trichomonas vaginalis* NYH286 cytadherence to HeLa cells and phenotype of high-molecular-weight immunogens (repertoire A).

Experiment no., subpopulation designation*	Cytadherence† (% of control)
1	
+/−	100
−	124
+	19
2	
+/−	100
−	122
+	53
3	
+/−	100
−	132
+	57

* Subpopulation designations were assigned previously after flow cytofluorometry and fluorescence-activated cell sorting with C20A3 monoclonal antibodies [16]. Plus (+) and minus (−) indicate presence or absence of fluorescence, respectively, on live organisms [15], and +/− represents heterogeneous nature of the parent NYH286 isolate.

† Cytadherence was determined by measuring the amount of radioactivity from radiolabeled trichomonads bound to HeLa cell monolayers [17]. Specific activities for each parasite sample were used to determine the number of organisms that attached to 5×10^4 HeLa cells. The ratio of parasites adherent to HeLa cells under the experimental conditions used ranged from 0.30 to 0.47 for parent NYH286. These values represented 100% for comparison with purified + or − subpopulations.

parts or the heterogeneous parent population. These data show the relation between the ability of trichomonads to cytadhere to HeLa cells and the A^- phenotype of the parasites.

Isolation of trichomonads on the basis of adherence phenotype and cytofluorometric analysis of adherent and nonadherent subpopulations. Earlier studies by us [22] and others [25] provided evidence for specific trichomonal parasitism of host cells. To facilitate and confirm identification of putative trichomonad adhesins, we wanted to isolate organisms from the parent *T. vaginalis* isolate that were incapable of parasitizing host cells [22, 23]. Purified nonadherent trichomonads also were grown in vitro for extended periods to monitor the reacquisition of the adherence phenotype. The original and all subsequently derived populations of *T. vaginalis* were monitored by flow cytofluorometry using a monoclonal antibody to a trichomonad immunogen of the A repertoire [16, 17, 21].

Figure 1 summarizes the cytofluorometric patterns and corresponding abilities of trichomonal populations to attach and kill HeLa cells. Only populations that possess A^- parasites (A, C, and D2) appear capable of killing HeLa cells. The isolated nonadherent subpopulation (B), for example, consisted of homogeneous A^+ trichomonads. Long-term cultivation of nonadherent organisms yielded a heterogeneous A^+

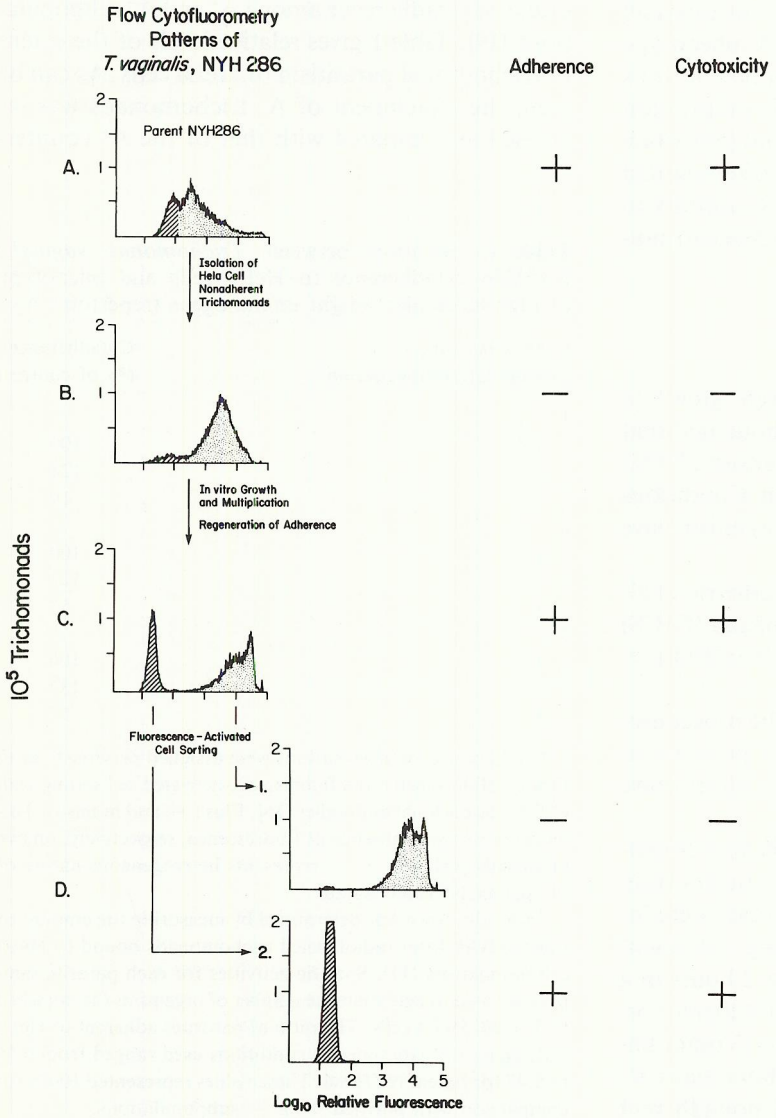

Figure 1. Relation between flow cytofluorometric patterns obtained with C20A3 monoclonal antibody [15, 16] and the ability of parent (A) and subpopulations (B, C, and D) of *Trichomonas vaginalis* to adhere to [17] and kill HeLa cells [18].

and A^- population of organisms that were adherent and capable of killing HeLa cells (part C). When heterogeneous populations were fractionated by FACS [19], only the trichomonads with the negative phenotype (A^-) possessed the ability to adhere to and mediate lysis of HeLa cells (D2 as compared with D1).

Recently, we identified four candidates for trichomonad adhesins (repertoire B) [23], and, as expected, only A^- trichomonads parasitizing HeLa cells (figure 1; A, C, and D) possessed adhesins (B^+). Nonadherent parasites did not synthesize the putative adhesins (B^-) nor did *Trichomonas tenax*, the nonpathogenic trichomonads of the oral cavity.

Discussion

Phenotypic variation may be coordinated and alternate between repertoires of specific *T. vaginalis* surface markers as follows: $A^+ B^- \leftrightarrow A^- B^+$. The significance of *T. vaginalis* phenotypic variation that involves alternating expression of repertoires of surface components is unknown. Our results, however, may be meaningful in light of the susceptibility of trichomonads to lysis by specific and nonspecific immune factors [18, 28]. However, it is important to point out that a recent study evaluating fresh isolates from patients with trichomoniasis suggested that most (80%) isolates may represent stable A^-B^+ phenotypes [20]. If this is true, it is uncertain what role, if any, the phenotypic variation as described here plays in parasite virulence and disease pathogenesis. Another interesting feature is that only isolates undergoing alternating expression of groups of proteins in trichomonal surfaces were infected with a double-stranded RNA virus [29].

Because *T. vaginalis* organisms of different phenotypes still have an extensive number of proteins in common [30], these observations do not rule out the possibility of developing immunodiagnostic tests based on antigen detection. The diversity among trichomonal isolates seen by immunofluorescence assays [12, 15, 17] reinforces the need to develop antibody reagents to common, stable and soluble immunogens found in patient secretions.

Our results point to interesting and unique features of the membranes of this pathogenic microorganism. It is clear that more work is necessary before we understand the mechanisms that regulate the placement of repertoires of molecules on the membranes of this pathogen and that may translate into the expression of virulence and pathogenesis of disease. This type of basic research may help us to eliminate or at least control this sexually transmitted disease agent by means of immunodiagnostic testing or the development of a vaccine.

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