

**TRICHOMONAS VAGINALIS PHENOTYPIC VARIATION
OCCURS ONLY AMONG TRICHOMONADS INFECTED WITH
THE DOUBLE-STRANDED RNA VIRUS**

BY ALICE WANG, CHING C. WANG, AND JOHN F. ALDERETE*

*From the Department of Pharmaceutical Chemistry, University of California School of Pharmacy, San Francisco, California 94143; and the *Department of Microbiology, University of Texas Health Science Center, San Antonio, Texas 78284*

Trichomonas vaginalis is a major sexually transmitted parasite that inhabits primarily the vaginal tract of women. Very little is known about precise virulence factors of the pathogenic human trichomonads, although recent reports (1–3) indicate that the ability of these parasites to undergo phenotypic variation may be related to expression of certain virulence attributes. For example, parasites that lacked a repertoire of major immunogens on their surface (negative phenotype) displayed enhanced cytoadherence and killing of host cells (3). On the other hand, trichomonads possessing surface immunogens (positive phenotype) had a diminished ability to cause contact-dependent cytotoxicity of HeLa cells; however, the positive phenotype organisms were still capable of shifting to the negative phenotype (3). More recent studies examining the phenotypes of fresh isolates of *T. vaginalis* (3, 4) confirmed the presence of only two types of parasite populations. On the basis of mAb reactivity via indirect immunofluorescence, isolates comprised homogeneous, negative phenotype trichomonads or heterogeneous populations containing both positive and negative phenotype trichomonads (3, 4). Isolates that were stable negative phenotype populations did not undergo phenotypic variation (3, 4). However, purified subpopulations of positive and negative phenotype trichomonads newly fractionated from the heterogeneous parents were both capable of phenotypic variation upon further in vitro cultivation (3, 4).

Another recent and as yet poorly understood observation that may be relevant to the biology of this host-parasite relationship is the presence of a virus-encoded double-stranded ribonucleic acid (dsRNA)¹ among some but not all isolates of *T. vaginalis* (5–7). The virus shown in Fig. 1 has an icosahedral morphology and a diameter of 33 nm. It consists of a single, linear dsRNA with an estimated size of 5.5 kb and a major protein of 85 kD. There is no detectable homology

This work was supported by Public Health Service grants AI-22380 to J. F. Alderete and AI-19391 to C. C. Wang. J. F. Alderete is a recipient of the Research Career Development Award K04-AI-00584. C. C. Wang is a Burroughs-Wellcome Scholar in Molecular Parasitology. Address correspondence to Dr. C. C. Wang, Dept. of Pharmaceutical Chemistry, School of Pharmacy, University of California at San Francisco, San Francisco, CA 94143.

¹ *Abbreviations used in this paper:* dsRNA, double-stranded RNA; MAR⁻, mAb resistant, negative phenotype; MAR⁺, mAb resistant, positive phenotype; TYM, trypticase-yeast extract-maltose medium.

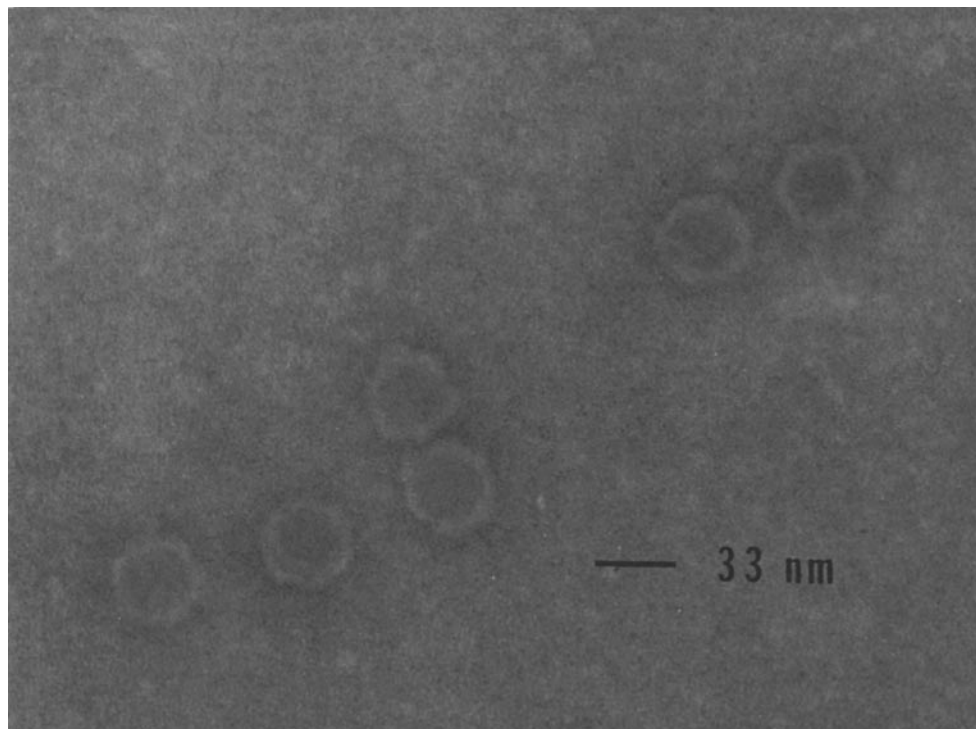


FIGURE 1. Electron micrograph of the purified dsRNA virus of *T. vaginalis*.

between the dsRNA and the DNA of infected host *T. vaginalis*. The virus appears to be closely associated with the membrane fractions of infected cells. Early examinations of various clinical isolates of *T. vaginalis* indicated that most of them contained the dsRNA virus ranging from 280 to 1,380 copies per cell. Interestingly, two metronidazole-resistant stains, IR78 and CDC85, were found to contain no detectable dsRNA (7). The initial correlation, however, between metronidazole resistance and absence of the dsRNA virus was proven coincidental by the recent finding that another *T. vaginalis* strain, RU375, which is sensitive to metronidazole (unpublished observations), had no detectable dsRNA.

The biological significance of the viral dsRNA within pathogenic human trichomonads is unknown. Interestingly, isolates such as IR78, CDC85, and RU375, which were stable negative phenotype when examined by indirect immunofluorescence and mAbs (3, 4), did not possess the dsRNA or the virus particles. This observation prompted us to examine whether only isolates of trichomonads that undergo phenotypic variation possess the viral dsRNA. In this report, we show that trichomonal isolates capable of phenotypic variation possess the dsRNA, whereas the absence of *T. vaginalis* surface expression of the major immunogen corresponds with loss of the viral dsRNA.

Materials and Methods

Isolates of *T. vaginalis* used in this study have been recently described (3–5, 8–10). Parasites were cultivated in Diamond's trypticase-yeast extract-maltose (TYM) medium

(11) supplemented with 10% heat-inactivated horse serum; growth properties have also been described (12). Only trichomonads at mid- to late-logarithmic phase of growth were used for all assays. Fresh isolates represent organisms grown axenically for no greater than 3–5 d after isolation (4). Long-term grown parasites were passaged daily in TYM-serum medium for six mo to two yr (1, 3, 10). Trichomonads were cryopreserved in liquid nitrogen until needed for designated experiments. Where necessary, fractionation of parent *T. vaginalis* populations was accomplished based on mAb reactivity (3) as described below, and subpopulations were cultivated and handled as above.

Indirect immunofluorescence and flow cytometry analysis of all isolates was recently performed for *T. vaginalis* (1–3). An mAb designated C20A3 was used for all fluorescent assays as described previously (3), and it is an IgG2a immunoglobulin directed toward a high-molecular-weight major immunogen that undergoes phenotypic variation (3). An example of negative and positive phenotype fluorescence corresponding to previously published flow cytometric data (3) generated for this study is presented in Fig. 2.

Rapid screening of all *T. vaginalis* cultures for the 5.5-kb dsRNA was done as detailed elsewhere (6). Briefly, 10 ml of late logarithmic-phase-growing trichomonads were pelleted, followed by vortexing for 5 s in 0.1 M sodium acetate, pH 5.0, and 1% sodium lauryl sulfate. The lysate was then extracted with unbuffered phenol at 65°C. An aliquot of the aqueous phase containing total cellular RNA was electrophoresed on 0.8% agarose, and the presence or absence of the dsRNA was determined by ethidium bromide staining. The dsRNA virus was also purified from selected isolates to reaffirm the relationship between the virus and the dsRNA (5). In all cases the absence of dsRNA among trichomonads resulted in no detectable virus particles in duplicate parasite preparations. All experiments were performed no less than three times using duplicate samples.

Results and Discussion

A total of 28 *T. vaginalis* isolates consisting of trichomonads grown for no more than 5 d in vitro versus parasites cultivated from 6 mo to >2 yr (1–5) have been examined for the presence of dsRNA (5–7). Only those isolates that contained trichomonads capable of undergoing phenotypic variation were found to possess the dsRNA. Table I summarizes representative data on the close relationship of the initial phenotypes of *T. vaginalis* isolates as determined by flow cytometry and indirect immunofluorescence assays (3) and the presence of dsRNA. Only those parent populations heterogeneous for the surface marker on the basis of mAb C20A3 reactivity possessed the dsRNA. Negative phenotype isolates were without dsRNA.

Table II presents a further examination of 15 representative fresh isolates. Initially, 11 isolates comprised heterogeneous populations for mAb reactivity, and all 11 possessed the dsRNA. After serial in vitro cultivation for several months, only three of the isolates (NYH286, JHH, and CzAL20W) had the dsRNA, and these isolates still represented heterogeneous populations based on flow cytometry (3). The remaining eight isolates lost the dsRNA upon long-term growth and comprised totally negative phenotype parasites. One of the isolates, MR100Cz, obtained from Dr. P. Demés of Comenius University, Bratislava, Czechoslovakia, was examined for the presence of virus-like particles by electron microscopy (5) before and after the long-term cultivations in our laboratory. The results indicated the presence of the virus-like particles in fresh isolate trichomonads, which disappeared upon prolonged in vitro transfers. These data strongly suggest that the loss of the viral dsRNA (5, 7) parallels the

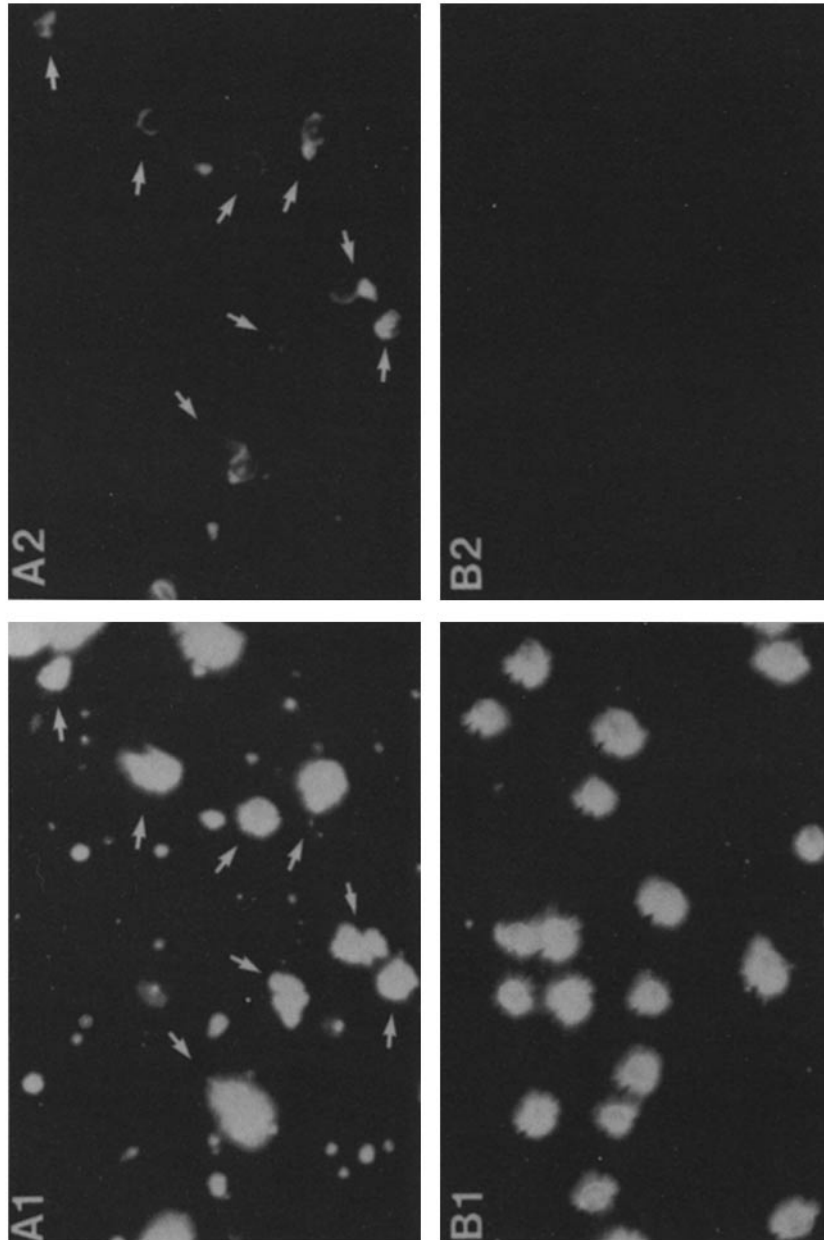


FIGURE 2. Dark-field (A1 and B1) and fluorescence (A2 and B2) microscopy with mAb C20A3 performed with fluorescence-activated cell sorted MAR⁺ (A) and MAR⁻ (B) subpopulations (3) of parent *T. vaginalis* NYH286. Flow cytofluorometric patterns for these purified subpopulations correspond with positive and negative fluorescence phenotypes as seen here for MAR⁺ (A) and MAR⁻ (B), respectively, and as recently reported (3). Arrows (A1 and A2) point to fluorescent trichomonads.

TABLE I
 Relationship between *T. vaginalis* Phenotypes and Presence of
 Trichomonad Virus dsRNA

| Isolate and subpopulation designation* | Extent of in vitro growth [†] | C20A3 phenotype [‡] | Presence or absence of dsRNA |
|--|--|------------------------------|------------------------------|
| JHHR | Fresh | +/- | + |
| ATCC30001 | Long-term | +/- | + |
| JH31A | Long-term | +/- | + |
| CDC85 [‡] | Long-term | - | - |
| IR78 [‡] | Long-term | - | - |
| RU375 | Fresh | - | - |
| DD3Cz | Fresh | - | - |
| 5147Cz | Fresh | - | - |
| AL8WCz | Fresh | - | - |
| AL11Cz | Fresh | - | - |
| 355Cz | Fresh | - | - |
| 427Cz | Fresh | - | - |
| JWCz | Fresh | - | - |

Determination of phenotype and presence of dsRNA was done as described in Materials and Methods. Phenotype refers to the expression of a specific surface marker by trichomonads assayed by indirect immunofluorescence or flow cytometry (3). dsRNA was based on ethidium bromide-stained gel patterns as seen in Fig. 1.

* Isolate designations are those used in prior reports as indicated in Materials and Methods.

[†] Trichomonads were grown in vitro in complex medium within 5 d after isolation (fresh) versus within extended periods of 6 mo to 2 yr (long-term), as indicated.

[‡] C20A3 phenotype refers to isolates that were either heterogeneous populations consisting of both positive and negative phenotype trichomonads on the basis of C20A3 mAb reactivity (+/-) or of isolates that were totally negative phenotype organisms (-).

[‡] Metronidazole-resistant strain.

absence of surface immunogens as well as the loss of phenotypic variation for the pathogenic human trichomonads (2, 3).

T. vaginalis such as NYH286 and JH31A have remained heterogeneous in parasite composition after long-term in vitro growth (Table II), and parasites from respective isolates undergo phenotypic variation (3). If the dsRNA presence is interrelated with the shift of positive to negative phenotype and vice versa (3), then both purified positive and negative subpopulations would be expected to harbor the dsRNA. Fluorescence-activated cell sorted subpopulations (3) designated mAb-resistant, positive phenotype (MAR⁺) and mAb-resistant, negative phenotype (MAR⁻), respectively, were obtained (Fig. 2) and examined for dsRNA content. Both MAR⁺ and MAR⁻ were found to have equivalent amounts of the dsRNA in agarose gels (Fig. 3), which was further illustrated by densitometer tracings of the individual dsRNA bands (LKB 2202 Ultrosan laser densitometer; LKB Instruments, Inc., Gaithersburg, MD). The data suggest the presence of about 2,400 copies of the dsRNA in every MAR⁺ or MAR⁻ trichomonad of NYH286 and 800 copies in every MAR⁺ or MAR⁻ trichomonad of JH31A.

Thus, it appears that viral dsRNA in *T. vaginalis* may allow for the surface

TABLE II
Loss of dsRNA and Phenotypic Variation Properties in T. vaginalis during Long-term In Vitro Growth

| Isolate and subpopulation designation* | C20A3 phenotype:* | | Presence or absence of dsRNA | |
|--|-------------------|----------------|------------------------------|-----------|
| | Fresh | Long-term | Fresh | Long-term |
| DD3 | - | - | - | - |
| AL11Cz | - | - | - | - |
| JW | - | - | - | - |
| 5147Cz | - | - | - | - |
| NYH286 | +/- [‡] | +/- | + | + |
| JHH | +/- | +/- | + | + |
| AL20WCz | +/- | +/- | + | + |
| 420Cz | +/- | - [‡] | + | - |
| 346Cz | +/- | - | + | - |
| AL10Cz | +/- | - | + | - |
| 8111Cz | +/- | - | + | - |
| 367Cz | +/- | - | + | - |
| 347Cz | +/- | - | + | - |
| AL8Cz | +/- | - | + | - |
| MR100Cz [‡] | +/- | - | + | - |

Fresh isolate trichomonads were grown in vitro in complex TYM medium supplemented with serum. The C20A3 phenotype of parasites was evaluated bimonthly by flow cytometry. Cultures were assayed at 3 mo for presence of the dsRNA.

* As described in Table I.

[‡] Metronidazole-resistant isolate.

disposition of prominent immunogens (1–3). This capability is lost upon in vitro cultivation of most clinical isolates of *T. vaginalis* along with loss of the dsRNA (Tables I and II). Electrophoretic analysis of 14 isolates originally harboring the dsRNA contained 400–1,200 virus particles per cell (data not shown).

We do not know the mechanism(s) by which the dsRNA within certain *T. vaginalis* may regulate or influence the surface expression of certain immunogens such as those recognized by the mAbs (2, 3). The loss of viral dsRNA and the concomitant absence of the surface disposition of specific immunogens (Table II) strengthens the likelihood of involvement of the virus in the sequestration of immunogens into trichomonad membranes. This is further reinforced by the detection of intrinsically labeled but not extrinsically labeled immunogen in the cytoplasm of negative phenotype trichomonads of isolates (2, 4) and subpopulations of isolates (3). Loss of virus, therefore, prevents expression of the C20A3 mAb-reactive immunogen on the parasite surface. This may be the first report showing that virus infection of protozoa induces important phenomena that may play a role in the host-parasite interrelationship (3). Finally, it is important to note that the stable negative phenotype that results from loss of the dsRNA virus represents a phenotype possessing established virulence properties, such as cytoadherence (13) and cytotoxicity (3, 13, 14) to epithelial cell surfaces.

Unfortunately, it has not been possible to infect the virus-free *T. vaginalis* organisms with the purified dsRNA virus (5) to further confirm the direct role, if any, of the dsRNA or dsRNA-directed products with the phenotypic variation

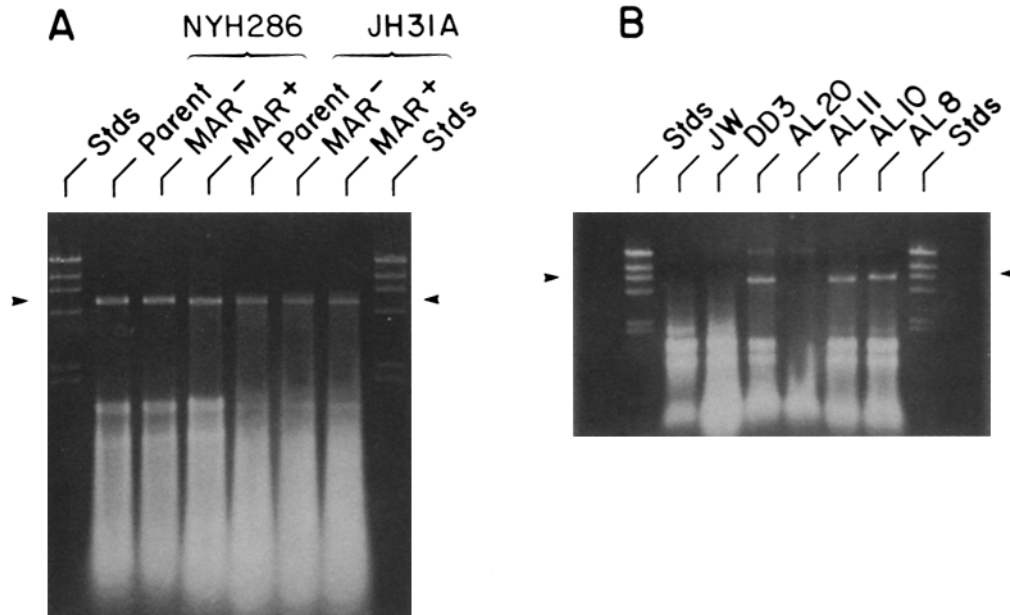


FIGURE 3. Demonstration of the presence of dsRNA among *T. vaginalis* isolates and subpopulations by agarose gel electrophoretic analysis of nucleic acid samples. (A) The parent *T. vaginalis* isolates NYH286 and JH31A as well as subpopulations fractionated with mAb C20A3. MAR⁻ and MAR⁺ refer to monoclonal antibody resistant, negative phenotype (nonfluorescent) and positive phenotype (fluorescent) trichomonads, respectively. (B) The presence and absence of dsRNA from nucleic acid samples of trichomonads of various fresh isolates. (Stds) Molecular weight standards included for comparative purposes. (Arrows) The presence of dsRNA. Each sample was derived from 2×10^7 trichomonads.

capabilities of *T. vaginalis*. The mAb C20A3, which detects a prominent immunogen with an M_r of $\sim 270,000$, was found to have no detectable crossreaction with a recently identified dsRNA viral protein of 85 kD (unpublished observation). Also, since some clinical isolates possess the dsRNA virus, additional research is necessary to evaluate the role, if any, of the virus and the pathobiology of the disease.

These data point to interesting and possibly important relationships between protozoa and viruses in terms of established virulence attributes (3, 13, 14). Thus, it is of interest to know whether *Giardia lamblia*, another pathogenic protozoan, has many of the features that have been described for the pathogenic human trichomonads (1, 3, 5), since this parasite has recently been found to harbor a similar dsRNA virus (6). Biological properties such as phenotypic variation (3) may be properties yet to be described for this and other pathogenic protozoa.

Summary

Trichomonas vaginalis isolates were examined for the presence of viral double-stranded RNA (dsRNA) and the property of phenotypic variation. Only the heterogeneous isolates composed of mAb-reactive and -nonreactive organisms, as determined by indirect immunofluorescence and flow cytometry, and

capable of phenotypic variation possessed the dsRNA. Both the positive and negative phenotype subpopulations separated from the heterogeneous parent contained equal amounts of the dsRNA. Loss of the dsRNA upon prolonged in vitro cultivation always correlated with the lack of expression of the major immunogen. The data indicate a relationship between the presence of the dsRNA and the ability of the pathogenic human trichomonads to express immunogens on their surfaces and to undergo phenotypic variation.

We gratefully acknowledge the excellent technical expertise of Guillermo E. Garza, Lorraine Kasmala, and Edmund C. Metcalfe. We appreciate the expert secretarial assistance of Mary Devadoss and Diana Hinojosa.

Received for publication 13 January 1987 and in revised form 23 March 1987.

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