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Experimental Parasitology 103 (2003) 44–50
Experimental Parasitology
www.elsevier.com/locate/yexpr

Trichomonas vaginalis: evaluating capsid proteins of dsRNA viruses and the dsRNA virus within patients attending a sexually transmitted disease clinic

J.F. Alderete,[a,*] K.A. Wendel,[b,d,1] A.M. Rompalo,[b,c,d] E.J. Erbelding,[b,d] M. Benchimol,[e] and T.-H. Chang[a]

[a] *Department of Microbiology, University of Texas Health Science Center, MC7758, 7703 Floyd Curl Dr., San Antonio, TX 78229-3900, USA*
[b] *Division of Infectious Diseases, Johns Hopkins University School of Medicine Baltimore, MD, USA*
[c] *Department of Epidemiology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, USA*
[d] *Baltimore City Health Department Sexually Transmitted Diseases Program, Baltimore, MD, USA*
[e] *RJ Universidade Santa Ursula, Rio de Janeiro, Brazil*

Received 7 October 2002; received in revised form 28 March 2003; accepted 10 April 2003

Abstract

Some isolates of *Trichomonas vaginalis*, the number one, non-viral sexually transmitted disease agent, are infected with one or several distinct double stranded (ds)-RNA virus. Immune rabbit anti-capsid serum (IRS) reacted with the capsid protein of purified dsRNA virus of a subset of the virus-infected *T. vaginalis* isolates. A monoclonal antibody (mAb) that recognized the capsid protein reactive with the IRS was generated. Analysis of the virus capsid protein of virus-infected isolates by probing nitrocellulose blots with mAb revealed diversity among immunoreactivity and in the size of the reactive capsid protein. Despite difficulties in visualizing virus within parasites by cross-section electron microscopy, gold-conjugated mAb readily labeled the cytoplasm of virus-positive trichomonads. Finally and importantly, isolates infecting patients attending an STD clinic, 75% of which were virus-positive isolates, had capsid protein of the same size detected by mAb present in all dsRNA viruses.
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Keywords: dsRNA virus; Patients; Sexually transmitted diseases; *Trichomonas vaginalis*; Urogenital; Vaginal

1. Introduction

The protozoan *Trichomonas vaginalis* causes the most prevalent non-viral sexually transmitted disease (STD) worldwide (WHO, 1995). Infection by this parasite causes severe adverse consequences for women and is associated with HIV transmission and adverse pregnancy outcomes. While much remains to be learned, some progress has been made in understanding virulence factors and aspects of disease pathogenesis (Alderete, 1999a,b; Petrin et al., 1998).

Interestingly, multiple distinct dsRNA viruses infect *T. vaginalis* (Flegr et al., 1988a, 1987b; Wang and Wang, 1985). This presence or absence of a dsRNA virus defines two naturally occurring types of isolates of *T. vaginalis* (Wang and Wang, 1986). The precise contribution of the virus to enhance or decrease virulence of this parasite is unknown, although the dsRNA virus influences the protein composition and growth kinetics of trichomonads (Provenzano et al., 1997). Numerous proteins are upregulated in expression within virus-infected trichomonads when compared with virus-minus progeny organisms (Khoshnan and Alderete, 1994; Wang et al., 1987). Similarly, there are numerous proteins expressed within the virus-minus progeny parasites that are absent within the virus-positive parental isolate trichomonads. Of particular interest is the fact that virus-infected organisms undergo phenotypic variation for a highly immunogenic protein P270 (Alderete et al., 1986a,b). These virus-infected parasites alternate between surface and cytoplasmic expression of P270, and a

* Corresponding author. Fax: 1-210-567-6612.
E-mail address: alderete@uthscsa.edu (J.F. Alderete).
[1] Current address: Oklahoma University Health Science Center, Division of Infectious Diseases, Oklahoma City, OK.

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doi:10.1016/S0014-4894(03)00068-7

relationship exists between the extent of P270 phosphorylation and surface localization (Alderete, 1999a,b). Further, *T. vaginalis* organisms with dsRNA virus have higher levels of *p270* transcription and correspondingly increased amounts of P270 (Khoshnan and Alderete, 1994). The virus-minus progeny trichomonads are unable to express P270 on the surface, and have lower amounts of P270 (Alderete et al., 1986a,b; Khoshnan and Alderete, 1994; Wang et al., 1987). Therefore, the virus appears to be involved in the modulation of expression of trichomonad proteins that could influence virulence attributes.

There exists divergence of the dsRNA genomes among the *T. vaginalis* viruses, and evidence supports the existence of at least three distinct dsRNA viruses (Tai et al., 1993). Although the dsRNAs encode for capsid and RNA-dependent RNA polymerase that are immuno-crossreactive (Bessarab et al., 2000; Liu et al., 1998), the dsRNAs do not cross-hybridize, consistent with significant non-identity of dsRNAs and encoded proteins at the nucleotide sequence level (Bessarab et al., 2000; Tai and Ip, 1995). In the study of *T. vaginalis* isolate T1 (Tai et al., 1993; Tai and Ip, 1995), Bessarab et al. (2000) demonstrated the existence of at least two similar-sized dsRNAs, showing that distinct dsRNA viruses infect one isolate. Importantly and supported by this study, the sizes of the capsid proteins encoded by the dsRNAs were different. Given the importance of the dsRNA virus to trichomonal biology, we generated a mAb to the capsid protein of the first dsRNA virus of isolate T1 that was studied (Liu et al., 1998). The mAb reacted with capsid proteins detected by immune rabbit anti-capsid serum (IRS) generated to the same T1 virus and recognized the capsid protein in other dsRNA viruses of infected *T. vaginalis* isolates. Analysis of purified virus confirms the existence of multiple viruses and capsid proteins of different sizes. Immunogold labeling using the mAb was reactive with the virus capsid protein in the cytoplasm of some virus-positive parasites. Finally, a capsid protein of the same size were detected by the mAb in all virus-harboring isolates of patients attending an STD clinic.

2. Methods

2.1. Trichomonal isolates and culture

Trichomonas vaginalis NYH 286 is a long-term-grown virus-harboring laboratory isolate (Alderete et al., 1986a,b; Wang et al., 1987). Isolates T068-II and 347 are virus-positive and T016 is virus-negative, which have been used before by us (Khoshnan and Alderete, 1993–1995; Wang et al., 1987). *T. vaginalis* 347N refers to agar-cloned, virus-progeny trichomonads derived from extended batch culturing of the parental 347 isolate (Khoshnan and Alderete, 1994). UT designations refer to fresh clinical isolates examined for this study. Twenty-one representative virus-harboring fresh isolates evaluated for reaction with mAb (Fig. 5) were from patients attending a sexually transmitted disease (STD) clinic and participating in a cross-sectional study, and details of this study were as recently published (Wendel et al., 2002).

2.2. Purification of dsRNA virus and dsRNA analysis

Purification of the dsRNA virus from sonicates using CsCl was performed as detailed previously (Khoshnan and Alderete, 1993). Approximately 2×10^9 PBS-washed trichomonads were suspended in 30 ml of TNM buffer (50 mM Tris, 150 mM NaCl, and 5 mM MgCl, pH 7.5). The mixture was freeze-thawed once then lysed by sonication for 2 min in an ice-cold condition. The lysate was clarified by centrifugation twice at 10,000 rpm for 20 min in a Sorvall SS 34 rotor. The supernatant was pelleted through a 20% sucrose cushion prepared in TNM buffer at 20,000 rpm in an SS 34 rotor for 2 h. The sediment containing the virus particles was suspended in 2 ml TNM buffer, equilibrated to a density of 1.35 g/ml with CsCl, and centrifuged at 25,000 rpm for 24 h in a SW 41 T1 rotor. Twenty fractions of 0.5 ml each were collected from the bottom of each tube and extensively dialyzed in TNM buffer. The fraction with the highest density was designated fraction 1. Fifty micro-liter of each fraction was mixed with 425 μl of double distilled, sterile water with the addition of 25 μl of 10% SDS followed by phenol–chloroform extraction. The RNA was precipitated with 3 vol of ethanol, and the dsRNA was examined by electrophoresis in a 1% agarose gel at 80 V for 2 h and visualized by ethidium bromide staining.

2.3. Generation of mAb 9A11 to capsid protein

The virus capsid protein used for immunization of mice to generate the monoclonal antibody was prepared by first pooling the dsRNA virus-containing fractions, which were then centrifuged at 20,000 rpm for 2 h in a SW 41 T1 rotor. The sediment was dissolved in 400 μl of electrophoresis dissolving buffer and boiled for 5 min. The sample was electrophoresed by SDS–PAGE on a 8% acrylamide separating gel. After protein separation, the acrylamide gel was briefly stained with Coomassie brilliant blue to locate the position of capsid protein of ~75 kDa (Liu et al., 1998). The band was excised, and the protein purified by electroelution. Finally, the eluted protein was dialyzed and the protein concentration adjusted to 200 μg/ml in PBS. Five BALB/c mice were immunized by subcutaneous injection with 50 μg of the antigen emulsified in Freund's complete adjuvant, and two additional boosts of the same amount of antigen

mixed with Freund's incomplete adjuvant were performed on days 14 and 28. A final boost of the same amount of antigen in saline was given intravenously at day 56, and the fusion was performed three days later.

The hybridoma producing mAb 9A11 (IgG_1) to the capsid protein was generated and examined using published protocols (Alderete et al., 1986a,b). ELISA screening of hybridoma supernatants detected the positive hybridoma supernatant. The assay was performed on microtiter wells coated with dsRNA virus purified as above. After incubating with hybridoma supernatant, reactions were followed by addition of alkaline phosphatase-conjugated goat anti-mouse IgG and color development. Reactive hybridomas were then single-cell cloned twice. Negative controls in all reactions included using supernatant from the myeloma cells used to produce the hybridomas, an irrelevant mAb of the same isotype, or secondary antibody.

2.4. Electrophoresis and immunoblot analysis of viral proteins and generation of mAb 9A11

The procedure for preparation of total trichomonad proteins for electrophoresis and immunoblotting is as detailed before (Khoshnan and Alderete, 1994). Total proteins from cell equivalents were always used throughout. All experiments were performed three separate times unless otherwise indicated. Proteins in electrophoresis dissolving buffer were boiled for 5 min before sodium dodecyl sulfate acrylamide gel electrophoresis (SDS–PAGE) in 8% acrylamide. Acrylamide gels with separated proteins were then stained with Coomassie brilliant blue and duplicate gels were blotted onto nitrocellulose by electroblotting in 20 mM Tris–HCl, pH 8.0,150 mM glycine, and 20% methanol. Membranes were treated with nonfat dry milk prepared in PBS prior to reacting with rabbit anti-capsid serum (IRS) or mouse mAb 9A11 directed toward the capsid protein (described below). A secondary goat anti-rabbit or anti-mouse IgG, respectively, conjugated to horseradish peroxidase was then added for development of protein bands. Normal rabbit serum (NRS) and irrelevant mAb of the same isotype were used as negative controls and were unreactive with the capsid proteins detected by IRS and mAb 9A11.

2.5. Immunocytochemical detection of dsRNA virus with mAb 9A11

The 33-nm icosahedral dsRNA virus can be visualized directly after purification of virus on CsCl gradients (Wang and Wang, 1986). This was accomplished by adsorbing purified virus onto Formvar-coated nickel grids or carbon-film prepared directly onto freshly cleft mica (Benchimol et al., 2002). Given only one report (Champney et al., 1995) on the difficulty in visualizing the dsRNA virus within trichomonads, we utilized the mAb for immunogold labeling of virus using standardized procedures (Alderete et al., 2001; Crouch et al., 2001). Briefly, parasites washed three times in PHEM buffer (50 mM $MgCl_2$, 70 mM KCl, 10 mM EGTA, 20 mM Hepes, and 60 mM Pipes, pH 6.8) at 37 °C were fixed overnight at RT in 100 mM sodium cacodylate buffer containing 0.4% glutaraldehyde, 4% paraformaldehyde, 0.5% sucrose, 1% picric acid, and 2 mM $CaCl_2$. After washing 3 times over a 15 min period, fixed organisms were dehydrated in ethanol prior to embedding in Unicryl (TedPella, Redding, CA, USA). Ultrathin sections of trichomonads processed as above were then collected in nickel grids followed by quenching with 50 mM NH_4Cl_2 with 3% BSA. The organisms were then washed in PBS-2% BSA, pH 8.0, for 30 min. Grids were then incubated with mAb 9A11 or control hybridoma supernatant. After incubation with antibody for 3 h at RT, the grids were washed prior to addition of 10 nm gold-conjugated goat anti-mouse antibody. The grids were finally stained in the dark for 20 min at RT with 5% uranyl acetate followed by lead citrate. Sections were then visualized by transmission electron microscopy (TEM) using a JEOL 1210 model microscope.

3. Results

3.1. Visualization of dsRNA virus proteins after SDS–PAGE and reactions of capsid proteins with IRS

Fig. 1a presents representative SDS–PAGE patterns of Coomassie brilliant blue-stained proteins readily visualized after electrophoresis of purified virus preparations obtained from identical cell equivalents from three virus-infected isolates. In each case, the same CsCl fraction that gave intense ethidium bromide-stained dsRNA bands in agarose gels (not shown) was used for SDS–PAGE. An intense band is readily seen that is consistent with that reported for the capsid protein (arrow) (Liu et al., 1998; Tai et al., 1993). The 160 kDa protein of isolates NYH 286 and 347 may correspond to the fusion protein of capsid and polymerase (Tai et al., 1993). Other less intense bands are visible for isolates NYH 286 and UT99-17 similar in size to the 75 kDa capsid protein (asterisk for NYH 286), possibly indicative of other dsRNA viruses with different-sized capsid proteins, as has been reported (Bessarab et al., 2000). The identities of lower M_r protein bands are unknown but may represent degradation products of the higher-sized proteins.

In the course of these experiments, we visualized a capsid protein for isolate T068-II (Fig. 1b(1)) that was greater in size than those seen in Fig. 1a. The mobility of the prominent capsid protein band was comparable to that seen for the band marked by the asterisk for NYH

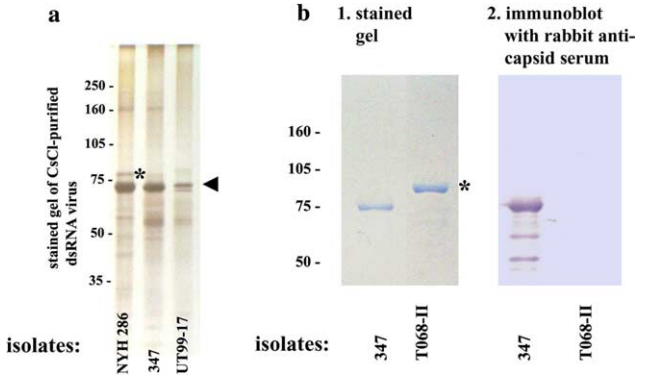

Fig. 1. Visualization of purified capsid protein and reactivity with IRS. (a) The SDS–PAGE patterns of Coomassie brilliant blue-stained proteins readily visualized after electrophoresis of purified virus preparations from identical cell equivalents of *T. vaginalis* isolates NYH 286, 347, and UT99-17, representative virus-harboring isolates. The arrow identifies the band of ~75 kDa consistent with the known size of the capsid protein (Bessarab et al., 2000; Tai and Ip, 1995). The asterisk for NYH 286 is indicated to show the possible existence of a distinct dsRNA virus with a capsid protein of a different size, as has been reported (Bessarab et al., 2000). (b) Nitrocellulose blots were probed with immune rabbit anti-capsid serum (Liu et al., 1998). Blots contained purified virus proteins after SDS–PAGE for the virus-harboring isolates 347 and T068-II. The numbers to the left of stained protein gels refer to molecular weight standards in kilodaltons.

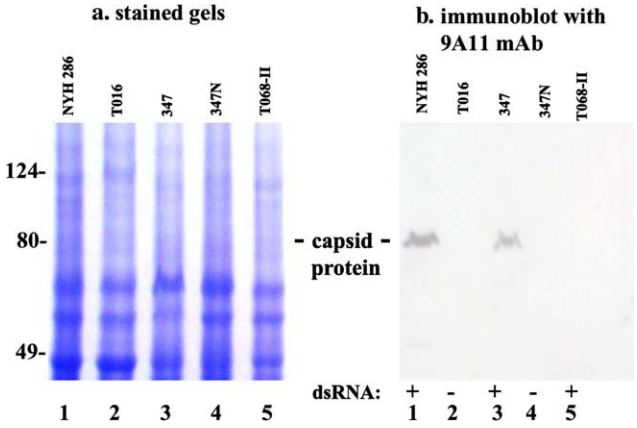

Fig. 2. Monoclonal antibody 9A11 detection of capsid protein from extracts of total *T. vaginalis* proteins. (a) Coomassie brilliant blue-stained total protein patterns ⩾40 kDa of *T. vaginalis* isolates showing equivalent amounts of protein loaded into individual wells. (b) Nitrocellulose blots of duplicate gels were probed with mAb. The designation 347N refers to virus-minus 347 progeny trichomonads derived from the virus-positive 347 parental organisms (Khoshnan and Alderete, 1994). The numbers to the left of stained protein gels refer to molecular weight standards in kilodaltons. dsRNA underneath the lanes in (b) indicates the presence (+) or absence (–) of ethidium bromide-stained dsRNA bands after 1% agarose gel electrophoresis of total nucleic acids.

286 (Fig. 1a). Immunoblots using immune rabbit anti-capsid serum (IRS) were performed on nitrocellulose blotted with the capsid proteins of isolates 347 and T068-II after SDS–PAGE of purified virus. As seen in Fig. 1b(2), the IRS readily detected the 75 kDa protein of dsRNA virus from 347 but not T068-II. The isolates NYH 286 and UT99-17 (Fig. 1a) gave results on blots as seen for isolate 347 (Fig. 1b(2)). The smaller mAb-reactive bands on blots likely represent degradation of capsid protein during the purification process. As a control, prebleed NRS gave no reactivity and clear duplicate blots similar to the blots for the T068-II reactions with IRS (Fig. 1b(2)) and as reported before (Liu et al., 1998). These results reaffirm the distinct immuno-reaction by IRS to capsid protein among dsRNA viruses of *T. vaginalis* and further suggest that the capsid proteins among viruses differ in size.

3.2. A mAb reacts with capsid protein in total trichomonad protein blots and shows size polymorphisms

We next generated a mAb that was reactive against purified capsid protein of virus of isolate 347. We tested for reactivity of nitrocellulose blots of total proteins of representative virus-harboring versus virus-minus *T. vaginalis* isolates. We included blots of proteins from virus-minus isolate 347 progeny trichomonads derived from the virus-positive 347 parental organisms (Khoshnan and Alderete, 1994). As expected and can be seen in Fig. 2b for total proteins >40 kDa, the mAb detected the capsid protein for virus-positive NYH 286 and 347 but not virus-positive T068-II. As expected, no band was detected for the virus-minus T016 and 347N. Fig. 2a is presented to illustrate the similar complex stained patterns of proteins showing equivalent amounts of samples in each lane and the ability to detect capsid protein in total protein blots.

We then purified dsRNA virus from other representative virus-positive isolates, including *T. vaginalis* T1 that contains two distinct dsRNA viruses with different-sized capsid proteins (Bessarab et al., 2000), one dsRNA virus of which is reactive with the original IRS (Fig. 1b(2)) (Liu et al., 1998). Stained gels after SDS–PAGE revealed one intense band (asterisk) and a minor band (dashed line) in the region of 75 kDa for some isolates (Fig. 3b). Immunoblots with mAb reacted only with the prominent band. These results reinforce the idea that the minor bands may represent a distinct virus capsid protein as reported earlier for isolate T1 (Bessarab et al., 2000). Further, these data suggest strongly the existence of size polymorphisms of capsid protein.

3.3. Immunocytochemical detection with mAb of dsRNA virus in the trichomonal cytoplasm

It has proven difficult to visualize the 33-nm dsRNA viruses within trichomonads and thereby obtain additional information regarding the virus and its cellular location. We, therefore, attempted to perform

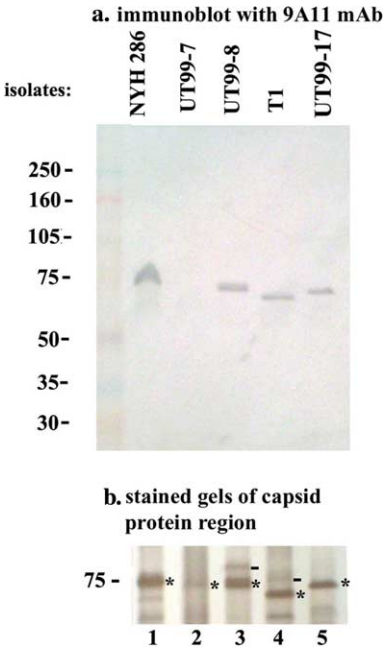

Fig. 3. Detection of purified capsid protein among representative virus-positive isolates *T. vaginalis* show size polymorphisms. (a) Nitrocellulose blots after SDS–PAGE of proteins from virus purified by CsCl from each isolate were probed with mAb. (b) The stained proteins of gels after SDS–PAGE illustrating the size polymorphisms that correspond with the mAb-reactive proteins in (a). Control hybridoma supernatant or irrelevant mAb of the same isotype gave no reactivity with virus proteins, and blots were like those seen for isolate UT99-7 in lane 2 (a). The numbers to the left of stained protein gels refer to molecular weight standards in kilodaltons.

immunogold labeling using the mAb. Gold labeling was apparent throughout the cytoplasm for isolate 347 in the same relative density as shown in Fig. 4b. There was no discernable aggregation of gold particles in specific sites within any part of the parasite. A cross-section electron photomicrograph is included in Fig. 4a to illustrate the complex structures evident throughout the cell and to show the integrity of the parasite during processing for immunogold labeling. There was no immunogold labeling evident for virus-minus isolate trichomonads handled identically. The dark bodies for the cross-section electron photomicrograph in Fig. 4a are the glycogen bodies found within the parasite.

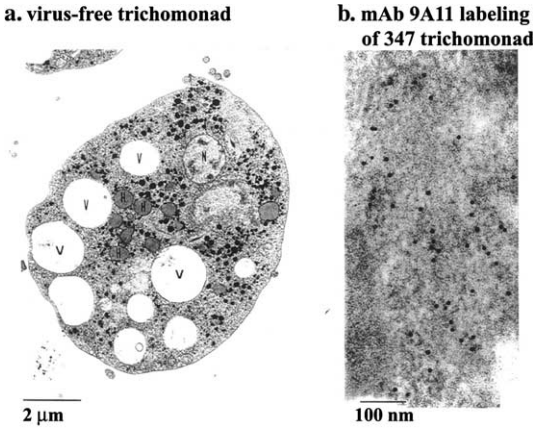

Fig. 4. Immunocytochemical detection of the dsRNA virus with mAb. (a) A general view of virus-minus *T. vaginalis* isolate T016. H, V, and N refer to the hydrogenosome organelles, vacuoles, and nucleus, respectively. (b) Immunogold detection of virus within the cytoplasm of *T. vaginalis* 347 by mAb 9A11, as described in Section 2. The labeling presented here is representative of that seen throughout the cytoplasm. Under no circumstances was any labeling evident at the same magnification for virus-minus trichomonads treated identically.

3.4. Analysis of capsid protein of dsRNA virus from trichomonads infecting patients attending a STD clinic

Twenty-one of 28 (75%) isolates of *T. vaginalis* from an STD clinic were virus-positive, and all had readily visible dsRNA in ethidium bromide-stained gels after agarose gel electrophoresis, as shown in Fig. 5a for representative isolates labeled 1 through 8. Isolate NYH 286 is virus-positive, and isolates T016 and 433 are virus-negative, which were included for comparison. Purified virus proteins were separated by SDS–PAGE, and capsid proteins of ~75 kDa were readily visible. Fig. 5 b and c are nitrocellulose blots prepared of duplicate

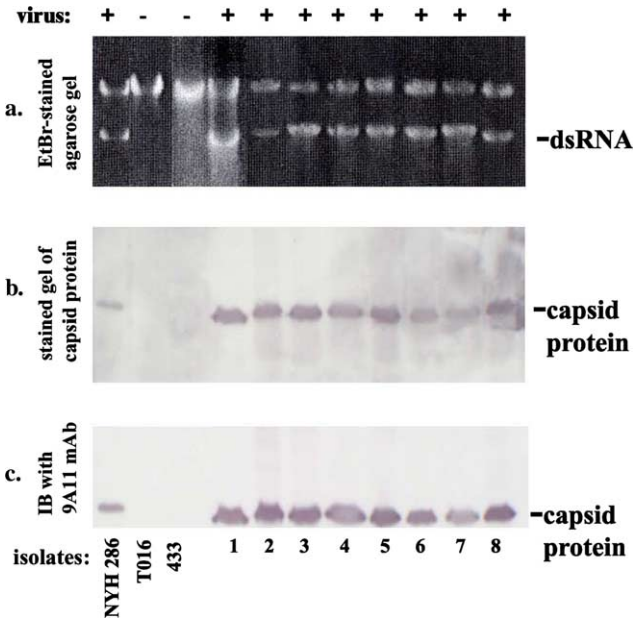

Fig. 5. Analysis of the mAb reactivity of purified dsRNA virus of infecting *T. vaginalis* isolates of patients attending an STD clinic. (a) The stained ethidium bromide-stained dsRNA bands in 1% agarose gels of total nucleic acids derived from eight of 21 isolates. The upper stained band represents trichomonal DNA, as shown before (Khoshnan and Alderete, 1993). Virus-harboring NYH 286 and virus-minus T016 and 433 isolates were included as controls. (b,c) Nitrocellulose blots of duplicate gels with the capsid proteins probed with IRS and mAb, respectively. Coomassie brilliant blue-stained protein gels gave protein band patterns of the capsid protein from purified dsRNA virus after SDS–PAGE identical to the blots, showing no additional proteins in this region.

samples after SDS–PAGE that were probed with rabbit anti-capsid serum and mAb, respectively, and all capsid proteins from the viruses from this patient population were immunoreactive. Identical results were obtained with both antisera and mAb. Importantly, stained protein gels gave protein bands identical to the blots, suggesting the absence of other possible viruses with different sized capsid proteins. These data indicate that the infecting cross-reacting virus of the isolates of *T. vaginalis* for this patient population may be related.

4. Discussion

The genome of the dsRNA virus of *T. vaginalis* encodes for capsid protein and RNA-dependent RNA polymerase (Bessarab et al., 2000; Tai and Ip, 1995). The genomic organization of the non-segmented genome yields a fusion protein of 160 kDa that comprises the capsid protein (~75 kDa) and polymerase (85 kDa). We confirmed earlier findings (Bessarab et al., 2000; Liu et al., 1998) that the capsid protein represents the most visible protein after electrophoresis of total purified virus proteins. In this study we demonstrate that the copy number of dsRNA virus is sufficiently high for detection of the capsid protein by a mAb in immunoblots with preparations of total trichomonad proteins (Fig. 2).

The mAb discriminated among dsRNA viruses on the basis of capsid protein reactivity, similar to that seen with the IRS of Liu et al. (1998). Importantly, stained gels of purified dsRNA virus of some isolates gave multiple bands of differing intensities in the region of the capsid protein (Fig. 3), suggesting the existence of distinct viruses within these isolates. In support of this, isolate T1 (Bessarab et al., 2000; Tai et al., 1993) has two distinct dsRNA viruses, and the viruses possessed capsid proteins of different sizes (Bessarab et al., 2000), consistent with the banding patterns reported here (Fig. 3, lane 4). Equally importantly, we show that a capsid protein detected by a mAb can vary in size. This finding is significant because it shows the genetic variations that exist within the capsid protein gene, and this may explain the diversity for this non-segmented dsRNA virus, as reported earlier (Tai et al., 1993).

While the 33-nm icosahedral dsRNA viruses have been readily visualized from purified CsCl fractions, there have been few reports on the detection of the dsRNA virus within trichomonads. One report examining terminal giant cells of a particular isolate of *T. vaginalis* showed photomicrographs of electron-dense particles resembling viral-like particles (VLPs) in the cytoplasm (Champney et al., 1995) and in inclusions in the nucleus. Attempts by us to visualize the dsRNA virus within the cytoplasm of infected trichomonads by electron microscopy have been unsuccessful. However, the availability of mAb made it possible to detect immunogold labeling in the cytoplasm of trichomonads prepared during the logarithmic phase of growth (Fig. 4). Importantly, the immunogold labeling was not seen in other organelles, indicating that the cytoplasm may be the replicative and natural site of the virus. It will be important in the future using a combination of antibodies to distinct viruses to examine whether they reside together or at distinct sites within the parasite.

Virus infection influences expression of numerous trichomonad proteins. The property of phenotypic variation, that is, the alternating surface versus cytoplasmic expression of the trichomonad protein P270 (Alderete et al., 1986a,b; Wang et al., 1987) and the adhesins (Alderete, 1988; Arroyo et al., 1992) occurs only among virus-harboring trichomonads. These earlier studies examined several isolates with viruses, and some like isolates NYH 286 and 347 were reactive with the IRS and mAb. The isolate T068-II is infected with a different dsRNA virus unreactive with these antibodies (Fig. 1) and also has phenotypic variation and properties similar to the other virus-infected isolates. Thus, this indicates that infection of *T. vaginalis* with any of the distinct dsRNA viruses permits changes of phenotypes and confers properties involved in virulence.

More recently, we have begun to ask whether patients infected with virus-positive isolates have symptomatology different from patients infected with virus-minus isolate trichomonads. Of interest would also be the proportion of patients in certain communities attending STD clinics with *T. vaginalis* either with or without dsRNA virus. We reported recently on a cross-sectional study done in an inner city STD clinic, where 75% of patients were infected with virus-harboring trichomonal isolates (Wendel et al., 2002). Interestingly, in this first study, no relationship with symptoms was observed in relation to the infecting isolate type. We now show that all dsRNA viruses had capsid proteins of the same electrophoretic mobility that were also reactive with mAb. It is tempting to hypothesize that the commonality of symptoms exhibited by these patients was because of similarities of the infecting isolates. This finding seems to reinforce the report by Laumann and Youm, 1999) on the patterns of sexual networks within this inner city group. Based on this report, it would be predicted that the infecting STD isolate would have common characteristics given the partner choices available within this population. If this were not the case, either capsid proteins unreactive with the mAb or reactive capsid proteins of different sizes might be predicted. Finally, as African Americans have higher infection rates for STDs, including *T. vaginalis* (Sorvillo et al., 2001), it would be of interest to examine other inner city populations attending STD clinics to determine the characteristics of the infecting *T. vaginalis* isolate. Clearly, the availability of reagents, such as this mAb to the dsRNA virus, permits the testing and perhaps

labeling of infecting isolates in order to make more accurate associations in the future regarding isolate type relative to disease outcomes.

Acknowledgments

We are grateful to Dr. J.-H. Tai of the Academia Sinica of Taiwan for the generous gift of rabbit anti-capsid serum against the dsRNA virus. The work in this paper was supported by Public Health Service Grants AI 43940 and AI 45429 from the National Institutes of Health (to JFA) and grants from Financiadora de Estudos e Projetos, Conselho Nacional de Desenvolvimento Cientifico e Tecnológico, Programa de Núcleos de Excelencia, Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro, PRONEX, and Associação Universitária Santa Úrsula (to MB). The assistance of Anna Lazzell of the Institutional Hybridoma Facility of the UTHSCSA is acknowledged. We are indebted to the staff of the Baltimore City Health Department Sexually Transmitted Disease Program (Baltimore). We thank Jeff Yuenger (Johns Hopkins School of Medicine, Baltimore) and Della Duncan (Baltimore City Health Department Sexually Transmitted Disease Program), for assistance and laboratory expertise. We are grateful to Dr. Jeanna Piper of the UTHSCSA for her assistance in obtaining the fresh clinical UT isolates used for comparative purposes.

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