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Trichomonas vaginalis: observation of coexistence of multiple viruses in the same isolate

Marlene Benchimol [a,*], T.-H. Chang [b], John F. Alderete [b]

[a] *Universidade Santa Úrsula, Rua Jornalista Orlando Dantas, 59, Botafogo RJ, CEP 22231-010, Brazil*
[b] *The University of Texas Health Science Center, San Antonio, TX 78284-7758, USA*

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Abstract

Trichomonas vaginalis is a flagellated, parasitic protozoan that inhabits the urogenital tract of humans. Some isolates of *T. vaginalis* are infected with a double-stranded RNA (dsRNA) virus, which was described in the literature as homogeneous icosahedral viral particles with an isometric symmetry and 33 nm in diameter. This study examined in detail the viral particles in *T. vaginalis* isolate 347 and describes a heterogeneous population of viral particles. The different dsRNA viruses were only observed after a change in the technique. The sample was prepared by the negative staining carbon-film method directly onto freshly cleft mica. The detected viruses ranged in size from 33 to 200 nm. Among the shapes observed were filamentous, cylindrical, and spherical particles. These results show that *T. vaginalis* may be a reservoir for several different dsRNA viruses simultaneously.

Keywords: Virus; Negative staining; *Trichomonas vaginalis*

1. Introduction

Trichomonas vaginalis is a parasitic flagellated protist that inhabits the urogenital tract of humans. This parasite is now the number one, non-viral sexually transmitted agent responsible for the disease trichomonosis [1]. Several reports have described the presence of double-stranded ribonucleic (dsRNA) viruses in different protozoa [2]. These dsRNA viruses have been found among some isolates of *T. vaginalis* [3,4], *Giardia* [5], *Leishmania* [6], and *Eimeria* [7]. These protozoa dsRNA viruses share several features. For example, the viral particles exhibit isometric symmetry and are 30–45 nm in diameter [8]. Except for *T. vaginalis*, only a non-segmented dsRNA viral genome was found within parasites. It has been shown that trichomonads may be infected by multiple distinct but related non-segmented dsRNA viruses [9]. Further, unrelated (non-hybridizing) dsRNA segments have also been demonstrated among the virus-harboring *T. vaginalis* organisms [10], suggesting either infection of trichomonads with a dsRNA virus with a tripartite genome or with at least three distinct, unrelated viruses.

One-half of *T. vaginalis* clinical isolates harbor a dsRNA virus [5,10], and the virus can be lost during batch cultivation, showing that infection permits environmental pressure for harboring of the virus or selection for virus-harboring trichomonads. Importantly, the presence of *T. vaginalis* dsRNA virus was found to correlate with the property of phenotypic variation defined as surface versus non-surface expression of a highly immunogenic protein termed P270 [11,12]. Recently, we demonstrated that large spherical viruses ranging in size from 83 to 104 nm were detectable in the cytoplasm and near the Golgi complex of dsRNA virus-positive trichomonads (M. Benchimol et al., submitted). That observation in addition to the existence of non-hybridizing dsRNAs within the virus-harboring organisms prompted us to consider alternative approaches for detection of different viruses. In this report, we now show that, by employing a modification in the negative staining preparation, indeed several different viruses that vary in shape and size were readily detected. This may be the first report that demonstrates that the dsRNA virus population within dsRNA virus-harboring parasites of *T. vaginalis* isolates may be more heterogeneous than previously thought.

* Corresponding author. Tel.: +55 (21) 2553 1615; Fax: +55 (21) 553 1615.
E-mail address: marleneben@uol.com.br (M. Benchimol).

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2. Materials and methods

2.1. Parasites

T. vaginalis organisms were grown in batch culture in complex Trypticase–yeast extract–maltose (TYM) medium supplemented with 5% heat-inactivated horse serum [13]. Parasites of isolate 347 are infected with the dsRNA virus and have three non-hybridizing dsRNAs of different sizes [12,14]. For some experiments, *T. vaginalis* 347 progeny organisms without virus and derived from the parental virus-infected parasites were used [12,14]. The virus-minus 347 trichomonads are referred to as 347v$^-$.

2.2. Detection of dsRNA in virus-harboring isolate trichomonads

Screening and rapid detection of dsRNA in total nucleic acid preparations was performed as detailed before [10,12].

2.3. Virus particle purification

Approximately 4×10^9 trichomonads were suspended in TNM buffer (150 mM NaCl, 5 mM $MgCl_2$, and 50 mM Tris, pH 7.5) and subjected to sonication until more than 90% of the cells were lysed. The lysate was clarified by centrifugation at least twice at $10\,000\times g$ for 20 min in a Sorvall SS34 rotor. The supernatant was then pelleted through a 20% sucrose cushion prepared in TNM buffer at $100\,000\times g$ in a SW40 rotor for 2 h. The sediment containing the virus particles was suspended in TNM buffer, equilibrated to a density of 1.35 g ml^{-1} with CsCl, and recentrifuged at $100\,000\times g$ for 24 h. Twenty-four fractions of 0.5 ml each were collected from the bottom of each tube and extensively dialyzed in TNM buffer. The fraction with the lightest density was designated fraction 1. One milliliter of each fraction was then treated with 50 μg ml^{-1} proteinase K and 1% sodium dodecyl sulfate (SDS) at 65°C for 30 min before phenol-chloroform extraction. The RNA was precipitated with three volumes of ethanol and separated in a 1% agarose gel, as described above. Fractions containing the viral dsRNA were further examined in a Jeol 1210 electron microscope.

2.4. Negative staining

Fractions containing the viral dsRNA were further examined for the presence of virus particles by dropping 1 μl of the virus fraction on a nickel grid coated with carbon. The virus sample was prepared by the negative staining carbon-film method directly onto freshly cleft mica. Grids coated with formvar, pilophorm or parlodium were also used. The negative staining was performed with 5% or 2% uranyl acetate for 1 min and observed in a Jeol 1210 electron microscope operating at 80 kV.

3. Results and discussion

In recent years virus-like particles have been found within numerous protozoan species. Successful infection and culture of purified parasite viruses in mammalian cells or infection of virus-negative organisms of the same species have been reported in very few protozoa [15]. Thus, it is not known if the viruses were acquired from the host in which the protozoan resided. In *T. vaginalis* an icosahedral 33-nm diameter dsRNA virus has been identified and characterized [3,4,9,16]. Previous studies demonstrated that virus-harboring trichomonads could have three unique non-hybridizing dsRNA segments with sizes ranging from 4.8 to 4.3 kb [10], possibly indicating the presence of a segmented virus or infection by different viruses. Other studies provided evidence for infection of trichomonads with divergent but related dsRNA virus simultaneously [9,16]. Further, small-sized satellite dsRNAs were found within many isolates of virus-infected *T. vaginalis* organisms [17,18]. In preliminary experiments, we found that the dsRNA genome was readily detected in a wide range of CsCl density centrifugation fractions. Therefore, we analyzed the various fractions with detectable dsRNA after CsCl density centrifugation derived from extracts of *T. vaginalis* isolate 347. The presence of any virus particles was examined by negative staining electron microscopy.

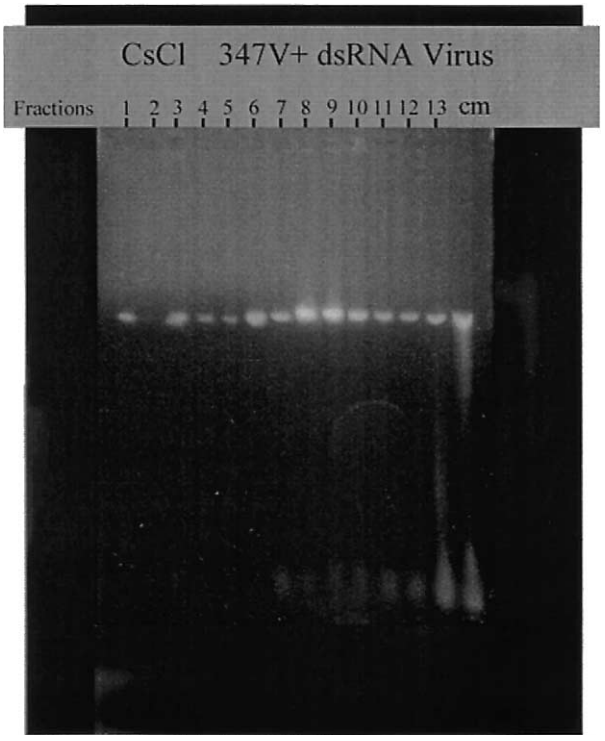

Fig. 1. Demonstration of virus dsRNA in virus-harboring trichomonad isolate 347. Nucleic acids of the virus-minus progeny 347 were examined for the presence of dsRNA as described in Section 2. The absence of dsRNA from the 347v$^-$ progeny is not shown. Bands were visualized by staining with ethidium bromide. Lanes 1 and 3–13 show dsRNA virus in *T. vaginalis* isolate 347.

Previous reports showed that at least five different sizes of dsRNA were found in various *T. vaginalis* isolates, suggesting that the coexistence of multiple dsRNA species is probably due to coinfection by heterologous viruses in the same cells [9,19].

Fig. 1 shows the presence of dsRNA among trichomonads of known virus-infected *T. vaginalis* isolates, such as the isolate 347. The EtBr-stained dsRNA was found in Fig. 1, lanes 8, 9 and 10 which were loaded with identical virus fractions. Likewise, no dsRNA was detectable under the same conditions for virus-minus progeny organisms derived from the virus-infected parental 347 isolate (not shown), as has been demonstrated previously [14]. The dsRNA derived from CsCl-purified virus [14,12] gave

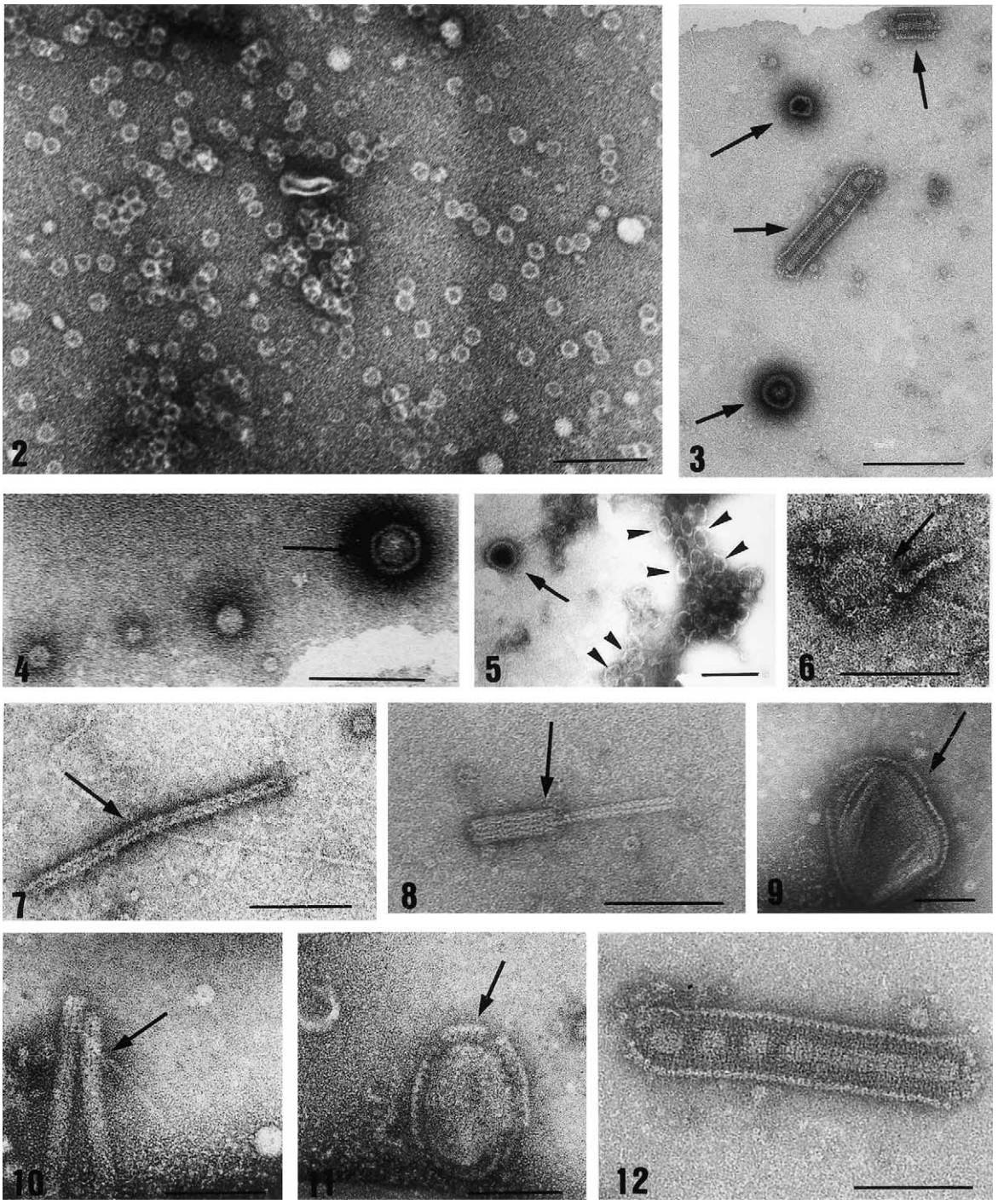

Fig. 2. Ultrastructural analysis of distinct dsRNA viruses in *T. vaginalis*. Electron micrographs of dsRNA-containing samples from fraction 7 of *T. vaginalis*, isolate 347^{+}, submitted to the CsCl buoyant density gradient centrifugation. Viral samples were adsorbed onto formvar-coated nickel grids (1) or carbon-film prepared directly onto freshly cleft mica (2–12) and negatively stained with 2% uranyl acetate. In 2, the population is formed by particles of 33 nm, whereas in 3–12 the virus sizes range from 33 to 200 nm (arrows). 2, 3, bar = 200 nm; 4–9, bar = 100 nm; 10–12, bar = 50 nm.

bands that migrated identically to the dsRNA in total nucleic acid preparations (Fig. 1), and no virus was ever detected within the virus-minus organisms. We then examined by electron microscopy (EM) three fractions with dsRNA, corresponding to Fig. 1, lanes 7–9, different virus-like particles based on size and morphologies were seen by negative staining, as described below. For improved observations, we modified slightly the procedure of preparation of samples for negative staining. In order to obtain higher contrast and avoid contamination by dirty materials, we first coated the grids with carbon-film sputtered twice on recently cleft mica, since mica sheets present many fresh, clean surfaces for this type of EM applications as carbon-filming. Next, the virus sample was dropped and stained with uranyl acetate for 1 min. The preparation obtained in this way was found to be clean, and several viruses presenting different sizes and shapes were clearly visualized (Fig. 2, 2–12). Importantly, the routine negative staining prepared on formvar-, pilophorm- or parlodium-coated grids allowed only the observation of the 33-nm icosahedral viruses. These viruses were found in all fractions, but were enriched on fraction corresponding to Fig. 1, lane 7. The observation that the other virus forms only after the carbon-film on freshly cleft mica was due to the lower number of the large-sized virus particles. Consequently, these results show that only very clean preparations as those from mica allowed us to readily visualize the new virus-like particles that exist within virus-harboring isolate 347 trichomonads.

When the carbon-film was used, virus-like particles with sizes varying from 33 to 200 nm and with shapes that were icosahedral, filamentous and cylindrical were observed (Fig. 2, 3–12). Regardless, the most frequent virus detectable was the icosahedral particle of 33 nm, which was seen with any coated grid, such as from the carbon-film method, formvar, parlodium or pilophorm (Fig. 2, 2). Importantly, the other forms and sizes were found only after carbon-film on freshly cleft mica (Fig. 2, 3–12).

The dsRNA virus of *T. vaginalis* was previously described as a uniform population of 33-nm diameter icosahedral particles, as seen in Fig. 2, 2 [5]. Since these observations, it has become clear that there exists a diverse family of related and similar-sized icosahedral dsRNA viruses capable of simultaneously infecting trichomonads [16,20]. Furthermore, the possibility of different unrelated viruses coinfecting trichomonads was demonstrated [10]. That a heterogeneous family of small-sized satellite dsRNAs were found within virus-harboring *T. vaginalis* trichomonads [17,21] reinforces the idea that coinfection by heterologous viruses is plausible. We recently demonstrated that different viruses were detectable within dsRNA virus-harboring organisms by thin sections. Their sizes varied from the original 33 nm (Fig. 2, 2) to large-sized 200-nm particles (Fig. 2, 3–12), and the most common shape in the thin sections was spherical, in the range size of 83 to 104 nm, although some icosahedral and oblong forms were also found. The spherical- and oblong-shaped virus-like particles had three distinct features: (1) an electron-dense core, (2) an electron-lucent middle part, and (3) an external electron-dense coat (M. Benchimol et al., submitted). These findings were performed in thin sections, where it is difficult to detect sectioned virus particles. In the present study we detected spherical, cylindrical, and filamentous virus particles that, to our knowledge, have not been described previously in trichomonads.

While the significance of our observations with respect to virulence and pathogenicity of *T. vaginalis* remains unknown, it is noteworthy that the possibility of trichomonads serving as a vector of other infectious agents of the upper genital tract has been reinforced through the findings showing mycoplasmas within trichomonads [22,23]. We now confirm the possibility of infection of parasites of an isolate of *T. vaginalis* by different viruses. Further, of interest is that the heterogeneous population of viruses as seen here are all simultaneously lost upon batch cultivation, as shown for the isolate used in this study [14,12]. Finally, studies are necessary to identify the taxonomy of these viruses and the role, if any, they play in the overall biology of the parasite and the host–parasite interrelationship.

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