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Mini review

Virus in Trichomonas—an ultrastructural study

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Abstract

Trichomonas vaginalis is a flagellated, parasitic protozoan that inhabits the urogenital tract of humans. Approximately one-half of isolates of *T. vaginalis* are infected with a double-stranded (ds) RNA virus, which was described in the literature as a homogeneous population of icosahedral virus with isometric symmetry and 33 nm in diameter. The present study describes the heterogeneous virus population found in *T. vaginalis* isolate 347. This population comprises different virus sizes (33–200 nm) and shape (filamentous, cylindrical, and spherical particles). These observations were made in CsCl-purified virus fractions as well as the thin sections of parasites. Some viruses were only observed after slight changes in the technique where the sample was prepared by the negative staining carbon-film method directly onto freshly cleft mica. The VLPs were found in the cytoplasm closely associated with the Golgi complex, with some VLPs budding from the Golgi, and other VLPs were detected adjacent to the plasma membrane. Unidentified cytoplasmic inclusions were observed in the region close to the VLPs and Golgi. These results indicate that *T. vaginalis* organisms may be infected with different dsRNA viruses simultaneously and suggest that *T. vaginalis* may be a reservoir for several viruses. We also showed some steps in the route of *T. vaginalis* virus and some aspects of the cytopathology of this infection. Purified VLPs were transfected to virus-free *T. vaginalis* isolates. Our results demonstrate that TVV attach and penetrate into trichomonads through endocytic coated pits and are maintained within vacuoles during batch culture for several daily passages. Immediately after virus transfection, many cells were lysed, whereas some intact reminiscent cells were recruited forming large clusters. Virus particles were found outside the cells, and in coated pits, within vacuoles in the cytoplasm, and infrequently within the nucleus. The Golgi complex showed changes in its electron density and in the cisternae structure. In lysed cells, virus particles were clearly seen over the residual membranes.

Keywords: *T. vaginalis*; Virus; Virus-like particles; Ultrastructure

Abbreviations: TVV, *Trichomonas vaginalis* virus; VLP, virus-like particles; TEM, transmission electron microscopy

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1. Introduction

Trichomonas vaginalis is a parasitic flagellated protist that inhabits the urogenital tract of humans (Plate 1). This parasite is now the primary, non-viral sexually transmitted agent responsible for the disease trichomonosis [1]. Several reports described the presence of dsRNA viruses in different protozoa [2]. These dsRNA viruses were found in several isolates of *T. vaginalis* [3,4], *Giardia* [5], *Leishmania* [6] and *Eimeria* [7]. Previous reports describe that these protozoan viruses share several features. For example, the viral genome is a non-segmented dsRNA, and viruses exhibit isometric symmetry and are 30–45 nm in diameter [8].

Half of the clinical *T. vaginalis* that were found close to the plasma isolates are persistently infected with a double-stranded RNA (dsRNA) virus [4,9]. Importantly, the presence of *T. vaginalis* dsRNA virus is related to surface expression and phenotypic variation of a highly immunogenic protein termed P270 [10,11], a property that occurs for a repertoire of high M_r proteins [10,12,13]. To date, no other biological property has been correlated between dsRNA virus infection and other protozoa.

2. Size and shape of virus heterogeneity

In the current literature, trichomonads viruses are described as a homogeneous population of icosahedral virus of isometric symmetry and 33 nm in diameter [Plate 1(2)]. However, recently we used a slightly modified negative staining preparation, using freshly cleft mica, and several different virus types in shape and size were detected, showing that the virus population is highly heterogeneous (Benchimol et al., submitted). The detected viruses ranged in size from 33 to 200 nm [Plate 1(2–7), Plate 2(9–13)]. Among the shapes observed were filamentous, cylindrical, and spherical particles. These results suggest that *T. vaginalis* may be a reservoir for several different dsRNA viruses simultaneously.

The TVVs are very difficult to observe in thin-sections. However, recently we were able to find large spherical viruses, having a size range of 83–120 nm on the cytoplasm and near the Golgi complex (Benchimol et al., submitted) [Plate 2(9–11)]. Their most common shape in the thin-sections was spherical, although some icosahedrical and oblong forms were also found. The spherical and oblong-shaped forms present three parts: (1) an electron-dense core; (2) an electron-lucent middle part, and (3) an external electron-dense coat [Plate 2(9–13)]. Some present a hollow core or a core of low electrondensity. These VLPs presented a preferential location: they are found over the cytoplasm, but mainly close to the Golgi complex [Plate 2(11,12)]. Some VLPs were even seen budding from the Golgi, whereas others were found very close to the membrane [Plate 2(9–11)]. Unidentified cytoplasmic inclusions were frequently observed in the region close to the VLPs and Golgi. Clusters of the already described icosahedrical virus were also observed in the cytoplasm, although less frequently (not shown).

3. Virus transfection

Previous attempts to establish persistent infection of virus-free *T. vaginalis* isolates have been unsuccessful [4]. It is worth noting, however, that *T. vaginalis* inoculated with genital HSV [14] internalised the virus into vacuoles, and this was followed by elimination of the virus. Further studies on the infectivity of CsCl-purified *T. vaginalis* dsRNA virus indicated an inability of the virus to infect other virus-free trichomonads, *Tritrichomonas foetus*, *Giardia lamblia*, *Entamoeba histolytica*, or *Trypanosonta brucei brucei* [2]. 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 many distinct, but related non-segmented dsRNA viruses [15]. Previous studies on *T. vaginalis* showed that virus-infected cells presented an unusual cytopathology, forming large cell masses by recruitment and aggregation of individual cells, which posteriorly lysed and died [8].

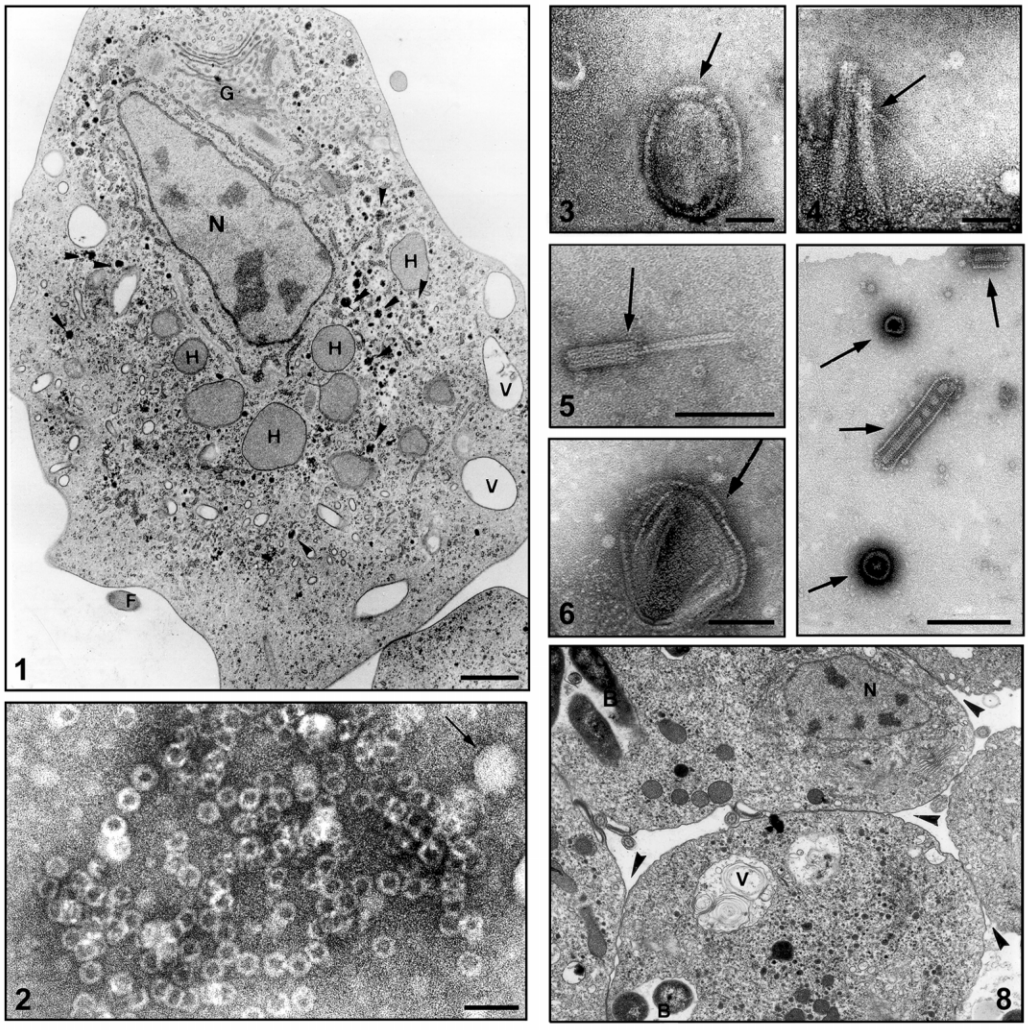

Plate 1. (**1**) General view of a virus-free *T. vaginalis*, isolate JT, in a routine thin section showing the overall organelles distribution, hydrogenosomes (H), nucleus (N), vacuoles (V). Note that no VLPs are observed, the Golgi (G) does not present dense bodies, and the dense particles distributed over the cytoplasm are glycogen granules (arrowheads). F, flagellum; ×15 000; bar = 650 nm. (**2–7**) 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 (2–7) or carbon-film prepared directly onto freshly cleft mica (2–7) and negatively stained with 2% uranyl acetate. In (2), the population is formed by particles of 33 nm, whereas in (3–7) the virus size range from 33 to 200 nm (arrows). Segment 2, bar = 100 nm; 3–7, bar = 50 nm; 5–6, bar = 100 nm; 7, bar = 200 nm. (**8**) *T. vaginalis*, isolate 347 V^{-}, after 1 h interaction with TVV. Sequential stages of the interaction between viral particles and trichomonads. The first observation is the formation of cell clusters, where the plasma membranes are closed apposed (arrow-heads). Bar = 140 nm.

Many independent isolates of *T. vaginalis* did not contain the virus, and concerning *Tritrichomonas foetus*, until today, are considered as virus-free. The lack of observations of the first steps of virus infection and its route in the parasite cell, led many authors to claim that this 33-nm virus is not transfected in vitro and the way of contamination remains a mystery [4]. As the *T. vaginalis*

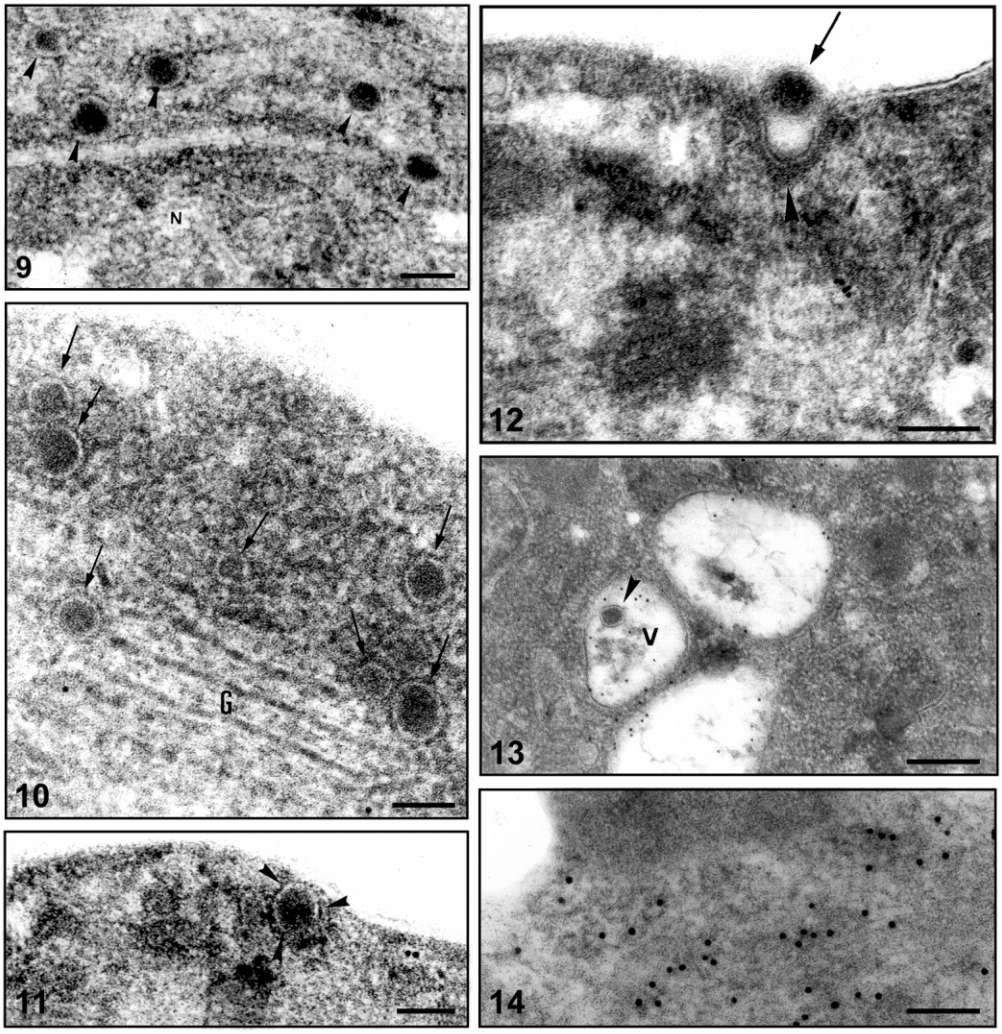

Plate 2. (**9–11**) *T. vaginalis,* isolate 347^{+}. Several VLPs (arrows), with sizes varying between 70 and 90 nm, are found in close association with the Golgi complex (G). They present a dense core, a middle electron-lucent layer and an outer coat. Notice that some particles are close to the nucleus [arrowheads in (11)] and to the cell surface (13); ×112 000, and bar = 90 nm. (**12–13**) Attachment of a virus to the parasite plasma membrane with subsequent internalisation through coated pits (12), and concentration of viruses in cytoplasmic vacuoles (13). The gold-conjugated lectin Coneanavalin A was used, but no labelling was found in the virus particle. Segment 12, bar = 100 nm; and (13), bar = 200 nm. (**14**) Immunolabeling of *T. vaginalis* virus in infected *T. vaginalis*. Trophozoites were infected with purified TVV and incubated at 37 °C for 1 h. Immunocytochemical reaction was performed using anti-347^{+} monoclonal antibodies and secondary antibody conjugated with 10-nm gold particles. Cytosol was labelled on specific regions. Bar = 100 nm.

virus (TVV) can be isolated from many isolates and can be used as a useful tool for studying the biology of this protozoan, we decided to shed some light on the infectivity process, in vitro.

Fractions containing the viral dsRNA were obtained by CsCl gradient, and were further examined for the presence of virus particles by negative staining with 5% uranyl acetate and also used in

transfection in *T. vaginalis* virus-free [Plate 1(2–7)]. Cells were examined by electron microscopy. Several cells were immediately lysed upon virus contact, whereas other cells were intact and formed large clusters [Plate 1(8)]. The plasma membranes were closely apposed [Plate 1(8)].

4. Trichomonads virus seems to enter T. vaginalis via endocytosis

We examined the entry of TVV into *T. vaginalis* trophozoites by electron microscopy. From this study we found a pathway consistent with endocytosis. In this pathway, TVV was seen adsorbed onto the plasma membrane and subsequently concentrated in vacuoles [Plate 2(12–13)]. Importantly, immunocytochemical studies showed the cytoplasmic presence of virus proteins, where perhaps viral replication takes place [Plate 2(14)]. This pathway resembles the uptake of exogenous lactoferrin observed in *T. foetus* [16]. Similar observations were made with *Giardia* virus [17]. These authors showed by electron microscopy that *Giardia lamblia* virus (GLV) particles were initially localised on plasma membrane, translocated to the peripheral vesicles and then spread to the cytoplasm. They also showed that inhibitors of endocytosis such as sodium azide, chloroquine, or ammonium chloride disrupted viral infection. In trichomonads, additional studies are necessary to confirm these results, but preliminary morphological observations indicate that both protozoa seem to engulf viral particles in a similar way.

Recently, Rendón-Maldonado et al. [18] showed that *T. vaginalis* interacts with HIV-1. The viruses were found on the parasite surface as well as in vacuoles even 2 days after in vitro interaction, suggesting that *T. vaginalis* may have a role in HIV transmission.

5. Cytophatic virus effect

T. vaginalis infected with a double-stranded RNA virus showed pronounced cytophatology ([8] and Pacheco et al, submitted). Immediately after the TVV transfection many cells were lysed, whereas some reminiscent cells were recruited forming large clusters [Plate 1(8)]. Virus particles were found outside the cells, in coated pits, within vacuoles in the cytoplasm, and not so frequently, in the nucleus. The Golgi complex showed changes in its electrondensity and in the cisternae structure. In lysed cells viral particles were clearly seen over the residual membranes ([8]; Pacheco et al., submitted). Clusters of electron-dense particles resembling viral structures in the cytoplasm were also seen.

6. Conclusions

We show that: (1) virus-free *T. vaginalis* can be transfected with CsCl purified dsRNA virus; (2) a transfected cell modified its cell structure and behaviour; (3) the route of virus acquisition in the trichomonad cell seems to be by endocytosis, through coated pits; (4) VLPs were found on the plasma membrane, vacuoles, cytoplasm and nucleus; (5) the virus population is comprised of different viral-like particles with varying sizes (33–200 nm) and shape (filamentous, cylindrical, and spherical particles); (6) the Golgi complex seems to be involved in the virus route, since VLPs were seen budding from Golgi cisternae.

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