Folia Microbiol. 32, 345–348 (1987)

The dsRNA of *Trichomonas vaginalis* Is Associated with Virus-Like Particles and Does Not Correlate with Metornidazole Resistance

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Received July 31, 1986

ABSTRACT. Twelve metronidazole-resistant and twelve metronidazole-susceptible strains of *Trichomonas vaginalis* were tested for the presence of dsRNA. Three resistant and five susceptible strains were found to contain dsRNA which indicated that metronidazole resistance does not correlate with the absence of dsRNA. Electron microscopy showed the homogenates of all dsRNA-positive strains to contain virus-like particles 32–38 nm in diameter, while no such particles were found in the dsRNA-negative strains. A mutual relationship between the dsRNA and virus-like particles seems to exist.

Some strains of *Trichomonas vaginalis*, a protozoan parasite living in the human urogenital tract, contain double-stranded RNA (dsRNA), often in amounts comparable to that of nuclear DNA (Wang and Wang 1985, Flegr *et al.* 1985).

The biological significance of dsRNA in trichomonads is not known. Its presence was shown not to be connected with virulence (Flegr *et al.* 1985). The results of Wang and Wang (1985), however, suggest a possible correlation between absence of dsRNA and resistance to metronidazole, a drug most often used in human trichomoniasis. Only two of the total of 35 strains tested did not contain dsRNA and they were the only metronidazole-resistant strains present in the sample. These results, together with the finding of a nuclear DNA sequence homologous with the dsRNA lead the authors conclude that dsRNA might be a physiological constituent of the *T. vaginalis* cell.

In contrast, Flegr *et al.* (1986) suggested a viral origin of dsRNA in *T. vaginalis* on the basis of the following observations.
1. dsRNA is found in some strains of *T. vaginalis* only. 2. The isolated nucleic acids contain several distinct populations of dsRNA molecules of various sizes, which resembles the situation in the segmented genome of dsRNA viruses. 3. The behaviour of dsRNA during fractionation of the homogenate using differential centrifugation suggests that it is particle-bound.

* After this paper had been accepted for publication the occurrence of virus-like particles in dsRNA-positive trichomonads was reported by others (Wang A.L., Wang C.C.: The double stranded RNA in *Trichomonas vaginalis* may originate from virus-like particles. *Proc.Nat.Acad. Sci.USA* **83**, 7956–7961, 1986).

In this paper we report the presence of virus-like particles in dsRNA-positive strains of *T. vaginalis** and the absence of any correlation between dsRNA and metronidazole resistance.

MATERIALS AND METHODS

Organisms. The strains employed are listed in Table I. All metronidazole-resistant strains were isolated from patients who were not cured by a standard treatment with metronidazole or other 5-nitroimidazole derivatives. The susceptible strains were isolated from patients cured by single standard metronidazole treatment (Entizol, *Polfa*, 250 mg three times a day for a 7 days). All strains were axenized and were maintained as cryostabilates (5 % dimethyl sulfoxide, liquid nitrogen). Their susceptibility to metronidazole

TABLE I. Strains used

Strains	Origin	Metronidazole MLC, μg/mL[a]	Presence of dsRNA
Resistant to metronidazole			
IR-78	Vienna, Austria (Meingassner and Thurner 1979)	100	–
BO	Gothenburg, Sweden (Forsgren and Forssman 1979)	50	–
Boston	Boston, USA (Müller *et al.* 1980)	25	–
Albany	Albany, USA (Müller *et al.* 1980)	25	–
Fall River	Fall River, USA (Müller *et al.* 1980)	100	+
MRP-2MT	Prague, ČSSR (Kulda *et al.* 1982)	100	–
SA	USA (Meingassner 1983)	79	+
141	*ditto*	50	–
316	*ditto*	100	–
NAD	Bratislava, ČSSR (Valent *et al.* 1985*a*)	–	+
K	Bratislava, ČSSR (Valent *et al.* 1985*b*)	70[b]	–
HL-2MT	Liberec, ČSSR (Kulda, *unpublished results*)	400	–
Sensitive to metronidazole			
A1	Vienna, Austria (Meingassner and Thurner 1979)	3.5	+
TV 5-27	Prague, ČSSR (Kulda *et al.* 1982)	2.5	–
TV 7-37	*ditto*	20	–
TV 10-02	*ditto*	3.1	+
TV 14-85	*ditto*	9.9	–
TV 17-48	*ditto*	12.5	–
TV 67-77	*ditto*	12.5	–
TV 73-87	*ditto*	3.9	+
TV 79-49	*ditto*	–	+
TV 85-08	Prague, ČSSR (Kulda, *unpublished results*)	6.3	+
TV 87-64	Prague, ČSSR (Kulda *et al.* 1982)	5.0	–
TV 99-51	*ditto*	12.5	–

[a]Mean of 3 independent estimations.
[b]Measured by P. Demeš (Bratislava), *personal communication.*

was determined by measuring the minimal lethal concentration (MLC) *in vitro* (Meingassner and Thurner 1979).

Cultivation. The strains were isolated according to Kulda *et al.* (1982) and grown in the trypticase, yeast extract, maltose (TYM) medium (pH 6.0) without antibiotics, containing inactivated horse serum (10 %) (Diamond 1957). The cells to be homogenized were cultivated in the medium without agar.

Susceptibility to metronidazole. The susceptibility was tested on microtitration plates according to Meingassner and Thurner (1979) in TYM medium without agar containing 10 % pre-colostrum calf serum (*Bioveta*, Ivanovice na Hané, Czechoslovakia). MLC was determined on the basis of loss of cell motility after a 48-h exposure to metronidazole under aerobic conditions.

Nucleic acid isolation. About 10^8 late-exponential-phase cells were washed with 0.8 % NaCl and extracted with phenol (pH 4.0) (Zasloff *et al.* 1978). The isolated nucleic acids contained only RNA (Plate 1). The precipitated nucleic acids were washed with ethanol and dissolved in 40 μL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Nucleic acid electrophoresis. The nucleic acids were separated in 1 % agarose using Tris-borate buffer (Maniatis *et al.* 1983); dsRNA of a type 3 human reovirus (strain Dearing) (Ramig *et al.* 1977) was used as standard.

Electron microscopy. Cell homogenates prepared by disintegration of *T. vaginalis* in a Potter-Elvehjem homogenizer were stored at −20 °C and negatively stained with phosphotungstic acid (Frank *et al.* 1978).

RESULTS

Twelve metronidazole-susceptible and twelve metronidazole-resistant strains were tested, of which only eight (five susceptible and three resistant) contained dsRNA. The mobility of dsRNA in agarose was the same in all positive strains (Plate 1).

Electron micrographs of cell homogenates of dsRNA positive strains showed numerous hexagonal particles (32—38 nm in diameter), uniform in size and shape (Plate 2). These structures resemble icosahedral virus-like particles. They were repeatedly found in all five dsRNA-positive strains but in none of the five dsRNA-negative strains tested.

DISCUSSION

In this paper we demonstrate the existence of dsRNA in a number of clinical isolates of *T. vaginalis*, confirming earlier reports (Wang and Wang 1985; Flegr *et al.* 1985) and the presence of virus-like particles in the homogenates of all dsRNA-containing isolates tested. This finding indicates that the dsRNA detected in this organism is of viral origin. The size (and shape) of the virus-like particles is similar to that of some dsRNA mycovirus capsids (Ushiyama 1985). Virus-like particles have been detected in a number of parasitic protozoa (Schuster and Durnebache 1971; Mattern *et al.* 1972) but their significance remains unknown.

Our results also confirm earlier reports which demonstrated that dsRNA is not present in all isolates (Flegr *et al.* 1986; Wang and Wang 1985) and show that virus-like particles are absent in organisms without dsRNA.

Our study included clinical isolates with various levels of metronidazole susceptibility. No correlation between this property and the presence of

dsRNA or of virus-like particles was found, however, in contrast to a report in which the absence of dsRNA was found to be correlated with metronidazole resistance (Wang and Wang 1985). A possible explanation for this discrepancy might reside in the selection of susceptible isolates by the latter authors which originated almost exclusively (31 of 33 isolates) from a single source (*National Defense Center*, Taipei, Taiwan) and thus may not represent independent strains. This could also explain the different frequency of the dsRNA-positive strains in the two samples (33 and 94 %, respectively).

The authors express their thanks to Dr. J.G. Meingassner (*Sandoz Research Institute*, Austria) for the strains of *T. vaginalis* isolated in Austria, Sweden and the USA, and to Dr. P. Demeš (*Institute of Parasitology, Comenius University*, Bratislava) for providing the strains isolated in Bratislava. The generous gift of reoviral dsRNA from Dr. M. Rosembergová (*Institute of Virology, Slovak Academy of Sciences*, Bratislava) is also gratefully acknowledged.

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The Plates will be found at the end of the issue.

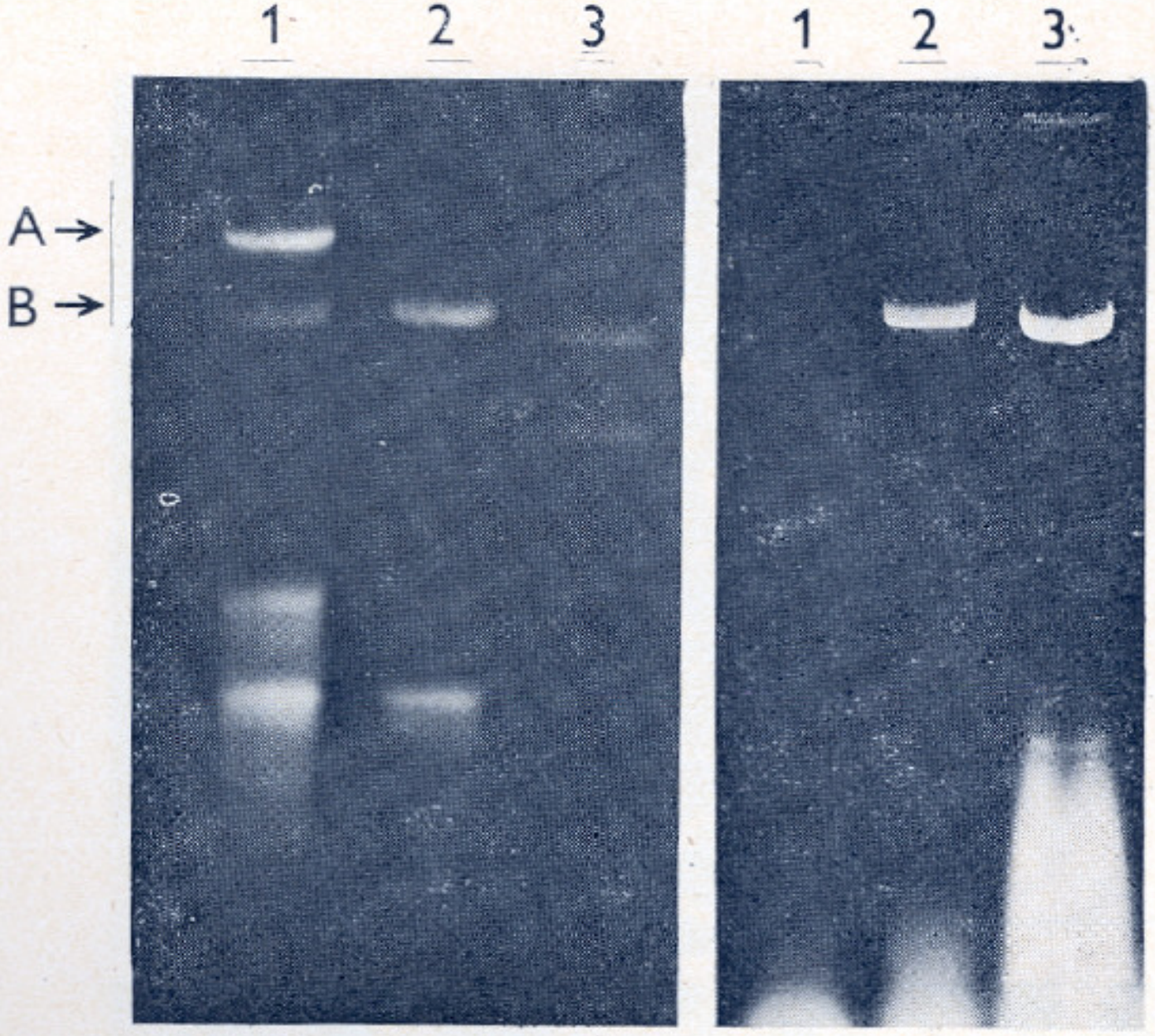

PLATE 1. Electrophoresis of nucleic acids of *T. vaginalis*; *left*: **1** — deproteinized with phenol at pH 8.1, **2** — deproteinized with phenol at pH 4.0, **3** — a standard of the type-3 reovirus dsRNA (strain Dearing), molar mass of the longest reoviral segment was 2.5 Mg per mol; *right*: **1** — strain BO, **2** —strain Fall River, **3** — strain TV 10-02 (strains BO and Fall River are metronidazole-resistant, TV 10-02 is metronidazole-sensitive), isolated with phenol at pH 4.0; **A** — DNA, **B** — dsRNA.

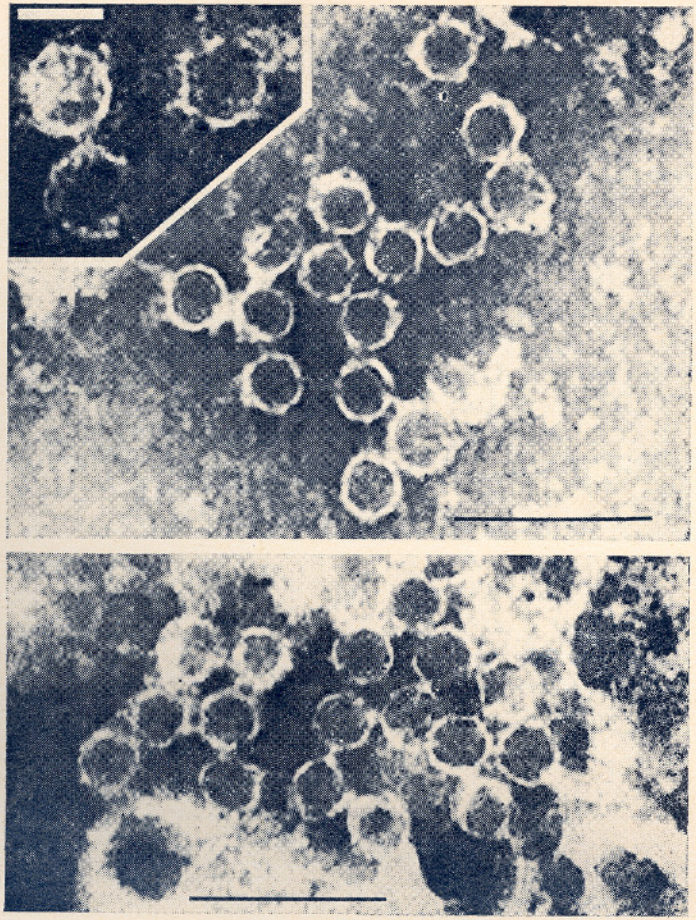

PLATE 2. Virus-like particles in cell homogenates of *T. vaginalis* strains containing dsRNA (negative staining by phosphotungstic acid); *black bars* represent 100 nm, *white bar* 30 nm.