

Hemolytic Activity of *Trichomonas vaginalis* and *Tritrichomonas foetus*

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The hemolytic activity of live isolates and clones of Trichomonas vaginalis and Tritrichomonas foetus was investigated. The isolates were tested against human erythrocytes. No hemolytic activity was detected by the isolates of T. foetus. Whereas the isolates of T. vaginalis lysed erythrocytes from all human blood groups. No hemolysin released by the parasites could be detected. Our preliminary results suggest that hemolysis depend on the susceptibility of red cell membranes to destabilization and the intervention of cell surface receptors as a mechanism of the hemolytic activity. The mechanism could be subject to strain-species-genera specific variation of trichomonads. The hemolytic activity of T. vaginalis is not due to a hemolysin or to a product of its metabolism. Pretreatment of trichomonads with concanavalin A reduced levels of hemolysis by 40%.

Key words: *Trichomonas vaginalis* - *Tritrichomonas foetus* - hemolytic activity - isolates - clones

Trichomonas vaginalis is a common cause of the infection of the female genital tract and trichomoniasis is recognized as a major sexually transmitted disease, while the *Tritrichomonas foetus* is responsible for the genital bovine urogenital trichomoniasis. However, the mechanisms of the pathogenicity of *T. vaginalis* and *T. foetus* have not yet been well defined. The pathogenicity of isolates of *T. vaginalis* and *T. foetus* have been previously reported, using mouse inoculation (Schnitzer et al. 1950, Bogovsky & Teras 1958, Honigberg 1961, Reardon et al. 1961, Frost & Honigberg 1962, Gobert et al. 1971, Dohnalová & Kulda 1975), growth characteristics (Kulda & Honigberg 1969, Kulda et al. 1970, Winston 1974), and also cytopathic effect assay (Brasseur & Savel 1982, Alderete & Pearlman 1984, Rasmussen et al. 1986, Roussel et al. 1991). Various systems have been developed to determine the hemolytic activity of *T. vaginalis* (Grys & Hernik 1973, 1974, Krieger et al. 1983, De Carli et al. 1989, Dailey et al. 1990, Potamianos et al. 1992), however, up to the present, the hemolytic activity of *T. foetus* has not been studied. The aim of this study is to determine a hemolytic activity of *T. foetus* and of *T. vaginalis*.

MATERIALS AND METHODS

Organisms - All strains of *T. vaginalis* (VG, GB, BoA, and Pc strains) studied were isolated from women with symptomatic vaginitis attending the Venereal Disease Department of the Charles Nicolle Hospital, Rouen, France. Four *T. foetus* strains used in this study (K, KV1, 5022, and PAL strains) were obtained from Prof. Wanderley de Souza (Institute of Biophysics, Federal University of Rio de Janeiro) and Helio Guida, DVM (EMBRAPA, Seropédica, RJ). The trichomonads were cultured axenically *in vitro* in trypticase-yeast extract-maltose (TYM) medium (Diamond 1957), supplemented with 10% heat inactivated cold horse serum at 37°C. The pH of TYM medium was adjusted to 7.0-7.2 for *T. foetus* and 6.0 for *T. vaginalis*. Isolates were subcultured every 48 hr in TYM medium. The strains were stored in liquid nitrogen (-196°C) with 5% of dimethyl sulfoxide (DMSO) (Warton & Honigberg 1980). The trichomonads in the logarithmic phase of growth and subcultured every 48 hr exhibited more than 95% mobility and normal morphology. The protozoa were counted with a hemocytometer and adjusted to a concentration of 1×10^6 living organisms per ml in TYM medium. Isolation of *T. vaginalis* clones followed the method recommended by Linstead (1989).

Erythrocytes - Fresh human blood was obtained at the City Emergency Hospital (HPS) blood center and also from volunteer donors. The blood was taken in an equal volume of Alsever's solution (dextrose 20.5 g, sodium citrate 8.0 g, citric acid

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0.55 g, sodium chloride 4.2 g, distilled water to 1 liter).

The erythrocytes were harvested and washed three times by centrifugation (250 x g for 10 min) in equal volume of Hank's balanced salt solution (HBSS) (Bio-Merieux, France). The supernatant was discarded. Each experiment was performed using fresh erythrocytes from all human blood groups. Whole human blood samples were previously examined and determined to be hepatitis B antigen (HBsAg) negative and human immunodeficiency virus (HIV-antibody) negative. The erythrocytes were stored at 4°C.

Hemolysis assay - The parasites were harvested from a 24 hr culture in TYM medium at 37°C and washed three times in HBSS by centrifugation (750 x g for 20 min). A volume of 50 µl of washed fresh undiluted erythrocytes was mixed with 2.5 ml of HBSS containing a total of 1×10^6 trophozoites of *T. vaginalis* or *T. foetus* (Krieger et al. 1983) originated from a 24 hr culture in TYM medium. After 18 hr of incubation at 37°C the mixture was centrifuged (250 x g for 10 min). Absorbance of the supernatant was measured at 540 nm (De Carli et al. 1989) with a spectrometer (Schimadzen UV 160) and was compared with a standard curve obtained by osmotic lysis of the erythrocytes of each species. Control tubes were included in all assays and the spontaneous hemolysis was also controlled. The results were expressed as percentage of total hemolysis (100%). The mean and the standard error of the hemolytic activity of every trichomonad species with the different erythrocytes were calculated after performing the assay at least 12 times in triplicate.

Lectins - Concanavalin A (Con A), from Sigma Chemical Co., St. Louis, MO, USA, was used in the concentration of 10 µg/ml diluted in phosphate buffered saline (PBS) 0.1 M pH 7.2. Equal volumes of trichomonads and Con A were incubated at 25°C under constant agitation. After 1 hr of incubation the flagellates were washed three times in 0.1 M PBS and harvested by centrifugation at 750 x g for 20 min (Warton & Honigberg 1980, Warton & Papadimitriou 1984). This experiment was done with human blood group type O.

RESULTS

T. foetus (K, KV1, 5022, and PAL strains) did not present any hemolytic activity against all human blood groups (Table I). The parasites maintained their mobility and did not show any morphologic abnormality in the tests without hemolysis. Also, no hemolysis was detected when *T. foetus* strains were tested against rabbit, rat and chicken erythrocytes.

T. vaginalis VG, Gb, Boa, and Pc strains hemo-

TABLE I

Hemolytic activity of four isolates and two clones of *Trichomonas vaginalis* and four isolates of *Tritrichomonas foetus* against human erythrocytes

Isolates	Number of assays	Hemolysis (%) ^a			
		Human erythrocytes			
		A	B	AB	O
VG	12	68±0.2	52±0.5	60±0.6	67±0.3
GB	12	92±0.2	75±0.2	76±0.2	96±0.2
Boa	12	76±0.3	57±0.3	76±0.2	76±0.7
Pc	12	70±1.0	52±1.1	65±1.0	66±0.6
C1	12	73±0.2	56±0.2	59±0.1	79±0.2
C2	12	65±0.2	59±0.3	56±0.2	72±0.3
K	12	NH	NH	NH	NH
KV1	12	NH	NH	NH	NH
5022	12	NH	NH	NH	NH
PAL	12	NH	NH	NH	NH

^a: the values represent the mean ± the standard error of triplicate samples. NH = no hemolysis.

lyzed all human blood groups (Table I), as well as rabbit, rat and chicken. All isolates tested presented a hemolytic activity from 52 to 96%. Hemolytic activity was maintained after a serial transfer in axenic culture for six months. The hemolytic activity varies according to donors origin of erythrocytes. No hemolysin released by the parasites could be identified.

Hemolysis did not occur with trichomonads culture supernatants from 24 and 48 hr kept at 37°C (Table II). Hemolytic activity was not observed with the hemolysis supernatant from 18 hr, and neither with sonicated extracts of trichomonads, and nor with previously killed organisms (Table II).

The hemolytic activity was reduced in 40% by the pre-treatment of *T. vaginalis* with Con A (Table III).

TABLE II

The effect of *Trichomonas vaginalis* culture supernatants from 24 and 48 hr, hemolysis supernatant from 18 hr, parasites sonicated extracts and killed organisms against human erythrocytes

Source ^a	Number of assays	Hemolysis (%)			
		Human erythrocytes			
		A	B	AB	O
Culture supernatants	12	NH	NH	NH	NH
Hemolysis supernatant	12	NH	NH	NH	NH
Sonicated extracts	12	NH	NH	NH	NH
Killed organisms	12	NH	NH	NH	NH

^a: culture supernatants from 1×10^6 trichomonads, hemolysis supernatant, parasites sonicated extracts and killed organisms were substituted for the trichomonads in the hemolysis assay. NH = no hemolysis.

TABLE III
Hemolytic activity of pretreatment strains of *Trichomonas vaginalis* with Concanavalin A against human erythrocytes of group type O

Treatment	Number of assays	Hemolysis (%) ^a			
		VG	GB	Boa	Pc
None	12	67±0.3	96±0.2	76±0.1	66±0.6
Con A	12	26±0.4	38±0.1	29±0.2	26±0.3

a: the hemolytic activity was determined as described in the materials and methods section. The values represent the mean ± the standard error of triplicate samples.

DISCUSSION

The hemolytic activity of some parasite protozoa was shown, particularly in *Trypanosoma congolense* (Tizard et al. 1977), *Trypanosoma brucei* (Tizard et al. 1978), *Entamoeba histolytica* (López-Revilla & Said-Fernández 1980) and *T. vaginalis* (Grys & Hernik, 1973, 1974, Krieger et al. 1983, Brasseur & Savel 1982, De Carli et al. 1989, Dailey et al. 1990, Potamianos et al. 1992), but probably the hemolytic activity does not follow the same mechanism in these different parasite species.

It was reported that the hemolytic activity of *T. congolense* is connected with the liberation of fatty acids by the action of a phospholipase A on the endogenous phosphatidyl choline (Tizard & Holmes 1976). The mechanism of this activity in *T. vaginalis* and *E. histolytica* has not yet been established. The strongest hemolytic potency in *E. histolytica* was observed in the most virulent strains (López-Revilla & Said-Fernández 1980). However, there is no correlation between this activity and an enterotoxin isolated from this amoeba (Lushbaugh et al. 1979).

Bacterial hemolysins have been reported (Freer & Arbutnott 1976) in staphylococci, streptococci, clostridia, vibrios, and aeromonas and have been confirmed to correlate with virulence in many species.

The hemolysis depends on the susceptibility of red blood cells membranes to destabilization (López-Revilla & Said-Fernández 1980).

Differences exist in different individuals of the same animal species in the susceptibility to a certain hemolysin (Cooper et al. 1966).

No enterotoxin was ever made evident (Brasseur & Savel 1982), and no hemolytic activity was observed with culture supernatants. Nevertheless, our results are not in opposition with the finding that hemolytic activity is dependent upon adherence of red blood cells to the surface of *T. vaginalis* (Potamianos et al. 1992).

Our preliminary results suggest that the hemolytic activity is not due to the hemolysin released by *T. vaginalis* or to a product of its metabolism. It is possible that the hemolytic activity remains in the dependence of parasitic and erythrocytic cell surface receptors which probably carry mannose, because this activity is strongly reduced by pre-treatment of the parasites with Con A. These data suggest the intervention of the cell surface receptors as a mechanism of the hemolytic activity. This mechanism could be subject to strain-species-genera specific variation of trichomonads.

A complete study of the activity of lectins and saccharides will allow the identification of specific receptors implicated in this activity.

However, *T. foetus* isolates do not present any hemolytic activity against rabbit, rat, chicken, and erythrocytes from all human blood groups. This is the most important point which requires further studies. Probably many mechanisms determine the pathogenic potential and hemolytic activity of the trichomonad trophozoites. It is essential that modern molecular characterization studies be conducted in conjunction with biological studies to determine the significance of hemolytic activity of *T. vaginalis*.

The recent isolation of intact *T. vaginalis* DNA (Riley & Krieger 1992) indicates the feasibility of further investigation and differentiation of these trichomonads using genetic engineering techniques.

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