Characterization of *Trichomonas vaginalis* haemolysis

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SUMMARY

The haemolytic activity of live *Trichomonas vaginalis* organisms was investigated. Optimal haemolysis of human erythrocytes was observed at a parasite to erythrocyte ratio of 1:5 during a 2 h incubation period. No haemolytic activity was detected in concentrated culture supernatants after overnight growth of trichomonads or when parasites were separated from erythrocytes by a 3 μm filter, suggesting a contact-dependent mechanism for haemolysis. The haemolytic activity was temperature-dependent and maximal haemolysis occurred at 37 °C. Treatment of trichomonads with metronidazole reduced levels of haemolysis by > 50%. Maximal haemolysis occurred at the pH range of the vagina during trichomoniasis. *N*-α-tosyl-L-lysyl-chloromethyl ketone and iodoacetamide, inhibitors of trichomonad cysteine proteinases, reduced the haemolytic activity of live parasites.

Key words: *Trichomonas vaginalis*, haemolytic activity, metronidazole, cysteine proteinases.

INTRODUCTION

Trichomonas vaginalis is a flagellated protozoan that causes sexually transmitted disease of the human urogenital tract. Clinical presentation of trichomoniasis can range from mild inflammation to a severe vaginitis with discharge, discomfort and sometimes pain. The exact mechanisms by which *T. vaginalis* parasitizes host vaginal tissues and produces disease are not known. Cytoadherence of the trichomonads to squamous vaginal epithelial cells has been demonstrated (Alderete *et al.* 1988) and represents an initial key step in infection. In addition, the trichomonads possess a contact-dependent cytolytic property active against human epithelial cells in culture (Alderete & Pearlman, 1984; Krieger, Ravdin & Rein, 1985; Pindak, Gardner & Pindak, 1986).

β-haemolysis by *T. vaginalis* has been reported, and correlation in levels of haemolytic activity with symptomatology among patients with trichomoniasis demonstrated, albeit with few isolates, but the parameters characterizing the haemolysis of *T. vaginalis* have not been established (Krieger, Poisson & Rein, 1983).

It is noteworthy that exacerbation of the clinical symptoms of trichomoniasis has been documented for patients during or immediately following menstruation (Brown, 1972; Jirovec & Petru, 1968). In some acute trichomoniasis cases, haemorrhagic spots have been described on the vagina leading to the description of a 'strawberry vagina' or 'scarlet fever vagina' (Honigberg, 1978). Interestingly, live trichomonads with adherent erythrocytes are occasionally observed in vaginal wet mounts from patients. Erythrocytes may well represent host cells targeted by the cytolysins of *T. vaginalis*, and investigations of the haemolytic activity of *T. vaginalis* may provide a model system for the study of these cytolysins and may shed light on unique features of this host–parasite inter-relationship which allow the parasite to survive *in vivo*.

In this report, we characterize the haemolytic activity of *T. vaginalis*. Metabolically active parasites were needed for efficient lysis of the erythrocytes, as has been demonstrated previously for cytotoxicity of epithelial cells in monolayer cultures (Alderete & Pearlman 1984, Krieger *et al.* 1985). Inhibitors of the cysteine proteinases of *T. vaginalis* (Coombs & North, 1983) greatly reduced or abolished haemolysis, implicating one or more of the trichomonad cysteine proteinases as potentiators of erythrocyte lysis. Importantly, a phenotypic variation was demonstrated in the levels of haemolysis caused by trichomonads of some isolates.

MATERIALS AND METHODS

Organisms

All *T. vaginalis* isolates have been previously characterized (Alderete & Garza, 1985; Alderete *et al.* 1987; Peterson & Alderete, 1982, Alderete *et al.* 1986*a*; Alderete, Suprun-Brown & Kasmala, 1986*b*; Arroyo & Alderete, 1989). Parasites were cultured at 37 °C in Diamond's trypticase–yeast extract–maltose (TYM) medium supplemented with heat-inactivated horse serum (Diamond, 1957). Only cultures containing motile trichomonads in the mid- to late-logarithmic phase of growth were used (Peterson & Alderete, 1982). Fresh clinical isolates were cultured for < 1 week.

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Table 1. The significance of contact between parasites and erythrocytes in haemolysis by *Trichomonas vaginalis* NYH 286

Experiment	Source	Haemolysis‖ (%)
1	Parasites*	81 ± 3·8
	Supernatant†	3 ± 0·8
	Conditioned PBS-maltose‡	2 ± 0·3
2	Parasites*	86 ± 2·7
	Parasites + 3 μm filter§	4 ± 1·2

* For this experiment 2×10^6 trichomonads were incubated with 1×10^7 erythrocytes at 37 °C.
† Culture supernatants from 4×10^8 parasites were concentrated 10-fold and substituted for the trichomonads in the haemolysis assay.
‡ For this experiment 2×10^6 trichomonads were incubated in PBS-maltose for 2 h and then removed by centrifugation. The erythocytes were then added to this supernatant and incubated as described in the Materials and Methods section.
§ In this experiment 2×10^6 trichomonads were separated from the 1×10^7 erythrocytes by Costar Transwell 3 μm pore-size filter units. No parasites were observed passing through the filter unit.
‖ Percentage haemolysis was determined as described in the Materials and Methods section. The values represent the mean ± the standard deviation of triplicate samples.

Table 2. The effect of pre-treatment of parasites with various agents and pH on haemolysis mediated by *Trichomonas vaginalis*

Experiment	Treatment*	Haemolysis† (%) Isolate: NYH 286	IR 78
1	None	76 ± 2·9	78 ± 1·2
	Mg^{2+} (0·2 mM)	80 ± 1·9	77 ± 2·9
	Ca^{2+} (0·2 mM)	74 ± 1·5	81 ± 0·7
	Metronidazole (10 mg/ml)	37 ± 0·6	33 ± 1·2
2	None	88 ± 1·0	92 ± 1·2
	Iodoacetamide (0·5 mM)	15 ± 1·4	22 ± 0·8
	TLCK (1 mM)	8 ± 0·5	11 ± 1·1
3	pH 5–6	81 ± 0·8	76 ± 1·5
	pH ⩾ 7	20 ± 2·8	20 ± 2·0

* Approximately 2×10^6 trichomonads were pre-incubated 30 min prior to addition of the parasite in the presence of reagent to erythrocytes for the haemolysis assay.
† Percentage haemolysis was determined as described in the Materials and Methods section. The values represent the mean ± the standard deviation of triplicate samples.

ratio needed for maximal levels of haemolysis. Optimal erythrocyte lysis by *T. vaginalis* NYH 286 was achieved at a ratio of 1 : 5 (parasites : erythrocytes), which corresponds to 2×10^6 parasites (data not shown). Table 1 presents data indicating the importance of contact for haemolysis by *T. vaginalis*. No haemolytic activity was detected in 10-fold concentrated culture supernatants from 4×10^8 organisms of either isolate NYH 286 or IR 78. Supernatants of trichomonads incubated at 37 °C for 2 h in PBS with maltose did not possess haemolytic activity. Haemolysis did not occur when a membrane with a 3 μm pore size was used to prevent contact between parasites and erythrocytes. These data suggest a need for contact for trichomonal haemolysis.

Biological properties of haemolysis

The addition of divalent cations such as magnesium and calcium to the incubation mixture did not alter haemolysis levels among the *T. vaginalis* isolates studied (Table 2). Moreover, pre-treating the trichomonads with 10 mM EDTA and the inclusion of EDTA in the haemolysis assay did not change the levels of haemolysis.

Metabolically active parasites were required for erythrocyte lysis, and similar energy-dependent cytolytic properties have previously been described for epithelial cell killing by *T. vaginalis* (Alderete & Pearlman, 1984, Krieger *et al.* 1985). Furthermore, a dependence for contact between live parasites and erythrocytes, as has been described for HeLa Cells, (Alderete & Pearlman, 1984) was also indicated by these experiments. Importantly, greater levels of haemolysis were obtained at pH values found in the vagina during infection (Jirovec & Petru, 1968; Honigberg, 1978) reinforcing the relevance of haemolysis as a property which may occur *in vivo*. Moreover, our data show that the haemolytic activity of *T. vaginalis* is not dependent upon the presence of divalent cations, which sets it apart from other microbial haemolysins (Rennie, Freer & Arbuthnott, 1974).

The vagina may be a suboptimal nutrient-deficient environment for maximal growth and multiplication of micro-organisms (Cohen *et al.* 1984). Although the significance of trichomonal-mediated haemolysis to the biology of this parasite or the host–parasite interface is not known, erythrocytes may represent a nutrient source for *T. vaginalis*. Trichomonads are deficient in lipid biosynthesis, and the erythrocyte membranes are rich in cholesterol (Nelson, 1967) that could be procured by the parasite. Furthermore, iron is an essential nutrient to the trichomonads and potentially could be acquired from the haemoglobin following haemolysis. These possibilities are reasonable given the facts that *T. vaginalis* has evolved exquisitely specific mechanisms by which to acquire essential nutrients. Receptors on the trichomonad surface bind apoprotein CIII of lipoproteins for uptake of lipids (Peterson & Alderete, 1984*a*), and iron accumulation results from receptor-mediated acquisition of lactoferrin (Peterson & Alderete, 1984*b*), the host iron-binding protein found in mucous secretions.

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