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Determination of Hemolytic Activity of Different Strains of Trichomonads of Genus *Trichomonas* Donné, 1836 and *Tritrichomonas* Kofoid, 1920

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ABSTRACT

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

INTRODUCTION

Trichomonas vaginalis is a common cause of the infection of the female genital tract; Trichomonas gallinae occurs in the upper digestive tract and in various organs of different avian groups, especially Columbiformes (doves and pigeons); Tritrichomonas foetus is responsible for genital bovine trichomoniasis while the Tritrichomonas suis occurs in the nasal cavity of domestic swine. The mechanism of pathogenicity of these trichomonads is not yet defined. Differences in virulence levels of the four strains have been evaluated using intraperitoneal and subcutaneous inoculations in mice (Schnitzer, Kelly and Leiwant 1950, Honigberg 1961, Frost and Honigberg 1962, Kulda 1965, Kulda and Honigberg 1969, Honigberg et al. 1970, Dohnalová and Kulda 1975). The hemolytic activity of T. vaginalis and its correlation with the virulence of strains has been previously described (Grys and Hernik 1973, Grys and Hernik 1974, Krieger, Poisson and Rein 1983, De Carli, Brasseur and Savel 1989, Dailey, Chang and Alderete 1990). However the cytopathogenic effect of T. gallinae, T. foetus and T. suis has not been studied, so far. The aim of this study was to determine the hemolytic activity of protozoan of genus Trichomonas and Tritrichomonas.

MATERIALS AND METHODS

Organisms: The T. vaginalis strain VG used in this study was isolated from a woman with symptomatic vaginitis attending the Venereal Disease Department of the Charles Nicolle Hospital, Rouen, France (Roussel, De Carli and Brasseur 1991). The T. gallinae strain G13 studied was obtained from the upper digestive tract of domestic pigeons Columba livia (De Carli, Pansera and Guerrero 1979) and T. suis strain SM93 was isolated from the nasal cavity of domestic swine Sus scrofa (Rott et al. 1993). Both isolations took place in the Laboratory of Parasitology, Pharmacy School of The Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil. T. foetus strain KV1 was received from Prof. Wanderley de Souza, Institute of Biophysics Carlos Chagas Filho of The Federal University of Rio de Janeiro, RJ, Brazil. 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 all trichomonads of veterinarian origin except to T. vaginalis; whose pH was adjusted to 6.0. Isolates were subcultured every 48 h in TYM medium. T. gallinae strains were cultured without antibiotics (penicillin and dihydrostreptomycin) (Stabler, Honigberg and King 1964). The strains were kept alive in liquid nitrogen (-196°C) with dimethyl sulfoxide (DMSO) (Warton and Honigberg 1980). The strains were kept in these conditions from the moment of isolation. At the time the experiments described in this report were done, all strains had been cultured for over 3 weeks after isolation. The trichomonads in the logarithmic phase of growth and subcultured every 48 h exhibited more than 95% mobility and normal morphology. The protozoa for the study were counted with a hemocytometer and adjusted to a concentration of 1×10^6 living organism per ml in TYM medium.

Erythrocytes. Fresh human blood was donated by the Town Emergency Hospital (HPS) and also by volunteer donators. The blood was taken in an equal volume of Alsever's solution. The erythrocytes were harvested by centrifugation at 250 x *g* for 10 min and washed three times with equal volume of Hank's Balanced Salt Solution (HBSS) with pH 7.2 (Bio-Merieux, France). The supernatant was carefully discarded. Each experiment was done using fresh erythrocytes from all human blood groups. The total human blood was previously examined and determined to be hepatitis B antigen (HBsAg) negative and human immunodeficient virus (HIV-antibody) negative in all collected blood. The erythrocytes were stored at 4°C.

Hemolysis assay. The parasites were harvest from a 24 h 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. gallinae or T. foetus or T. suis (Krieger, Poisson and Rein 1983) originated from a 24 h culture in TYM medium. After 18 h of incubation at 37°C the mixture was centrifugated at 250 x *g* for 10 min. The absorbance of the supernatant was measured at 540 nm (De Carli, Brasseur and Savel 1989) and was compared with a standard curve obtained by osmotic lysis of 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 (S.E.M) of the hemolytic activity of every trichomonad species with the different erythrocytes were calculated after performing the assay at least 24 times in triplicate.

Lectins. Concanavalin A (Con A), from Sigma Chemical Co., St. Louis, Mo, USA, was used in the concentration of 10 mg/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 h of incubation the flagellates were washed 3 times in 0.1 M PBS and harvested by centrifugation at 750 x *g* for 20 min. (Warton and Papadimitriou 1984). This experiment was done with human blood group type O.

RESULTS

All experiments in this study were performed within 6 months. T. foetus KV1 strain and T. suis SM93 strain did not present any hemolytic activity against all human blood groups (Table 1). The parasites maintained their mobility and did not show any morphologic abnormality in the tests without hemolysis. T.

vaginalis VG strain and T. gallinae G13 strain hemolyzed all human blood groups (Table 1). All isolates tested presented an hemolytic activity from 31 to 68%. The hemolytic activity was maintained after a serial transfer in axenic culture for 6 months. The hemolytic activity varies according to donators origin of erythrocytes. No hemolysin released by the parasites could be identified. Hemolysis did not occur with trichomonads culture supernatants from 24 and 48 h kept at 37°C (Table 2). The hemolytic activity was not observed with the hemolysis supernatant from 18 h and neither with sonicated extracts of trichomonads and nor with previously killed organisms (Table 2). The hemolytic activity was reduced in 40% by the pre-treatment of T. vaginalis VG and T. gallinae G13 with Con A. (Table 3).

DISCUSSION

The hemolytic activity of some parasites protozoa was shown, particularly in Trypanosoma congolense (Tizard et al. 1977), Trypanosoma brucei (Tizard, Sheppard and Nielsen 1978), Entamoeba histolytica (López-Revilla and Said-Fernández 1980) and T. vaginalis (Krieger, Poisson and Rein 1983, De Carli, Brassuer, Savel 1989, Dailey, Chang and Alderete 1990), but probably the hemolytic activity does not follow the same mechanism in these different parasites. 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 and 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 and Said-Fernández 1980). However there is no correlation between this activity and an enterotoxin isolated in this amoeba (Lushbaugh et al. 1979). Bacteria hemolysins have been reported (Freer and Arbuthnott 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. Differences exist in different individuals of the same animal species in the susceptibility to a certain hemolysin (Cooper and Madoff 1966). No enterotoxin was never made evident (Brasseur and Savel 1982) and no hemolytic activity was observed with culture supernatants. These results suggest that the hemolytic activity is not due to hemolysin released by T. vaginalis VG and T. gallinae G13 or to a product of its metabolism. It is possible that the hemolytic activity remain in the dependence of parasitic and erythrocytic cell surface receptors which are carriers of o-methyl-mannoside and/or n-acetyl-glucosamine, 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-genera-species specific variation of trichomonads. A complete study of the activity of lectins and saccharides (Roussel, De Carli and Brassuer 1991) will allow the identification of specific receptors implicated in this activity. Probably many mechanisms determine the pathogenic potential of the trichomonads trophozoites. The recent isolation of intact T. vaginalis DNA (Riley and Krieger 1992) indicates the feasibility of further investigation and differentiation of these trichomonads using genetic engineering techniques.

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Table 1. The hemolytic activity of trichomonads of genus Trichomonas and Tritrichomonas against erythrocytes from all human blood groups

Isolates	Number of assay	Hemolysis (%)* Erythrocytes A	B	AB	O
VG	24	68±0.2	52±0.5	60±0.6	67±0.3
G13	24	35±2.4	34±2.4	31±3.1	33±2.2
KV1	24	NH	NH	NH	NH
SM93	24	NH	NH	NH	NH

*The values represent the mean ± the standard error of triplicate samples. NH = No hemolysis.

Table 2. The effect of trichomonads culture supernatants from 24 and 48 h, hemolysis supernatant from 18 h, parasites sonicated extracts and killed organisms against erythrocytes from all human blood groups

Source*	Number of assay	Hemolysis (%) 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

*Culture supernatants from 1 x 10^6 trichomonads, hemolysis supernatant, parasites sonicated extracts and killed organisms were substituted for the trichomonads in the hemolysis assay. NH = No hemolysis.

Table 3. The hemolytic activity of pre-treatment strains of T. vaginalis and T. gallinae with Con A against human blood group type O

Treatment	Number of assay	Hemolysis (%)* Isolates VG	G13
None	24	67±0.3	33±2.2
Con A	24	26±0.4	13±0.4

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

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