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Iron mediates *Trichomonas vaginalis* resistance to complement lysis

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Trichomonas vaginalis, a sexually transmitted disease agent in humans, is readily lysed by activation of the alternative complement pathway. The parasite became resistant following growth in medium supplemented by iron compared to parasites grown in medium depleted of iron, which were readily killed by complement. The resistance to complement was dependent on iron concentration while divalent cations other than iron were ineffective, showing specific regulation of this property by iron. Lactoferrin, but not transferrin, rendered low-iron parasites resistant to complement lysis, reinforcing the *in vivo* modulation by a known source of iron for this parasite. Pretreatment of high-iron, complement-resistant parasites with proteinase inhibitors resulted in lysis by complement, indicating that resistance was likely due to proteinase degradation of C3 on the trichomonal surface.

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Introduction

Trichomonas vaginalis, mucosal protozoan parasite of the urogenital tract, is responsible for the most common clinically recognized sexually transmitted disease. This pathogen survives in the vagina of women, an environment that undergoes profound changes during the menstrual cycle. During menstruation, the vaginal microenvironment changes suddenly and dramatically with serum proteins, erythrocytes and numerous other blood macromolecules flooding the vaginal epithelium. It is at this time that *T. vaginalis* encounters complement *in vivo*.[1]

Activation of complement has the potential to be an important effector system against *T. vaginalis* infection. The alternative pathway is independent of antibody and requires Mg^{2+}, factors B and D, properdin and C3 for activation. The considerable lytic activity of fresh, non-immune human serum on *T. vaginalis* has been observed by numerous investigators.[2–5] These observations have been recently extended in a study showing that menstrual blood complement *in vivo* is trichomonicidal.[6] Lysis

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is due to the activation of the alternative pathway by trichomonads in the absence of specific antibodies.[2]

A recent investigation demonstrated that fresh isolates of *T. vaginalis* differed in their susceptibility or resistance to complement-mediated lysis in serum.[1] Especially noteworthy was the observation of complement-resistant isolates becoming susceptible to complement-mediated lysis after extended *in vitro* cultivation.[1] These data are consistent with the hypothesis that the surface of trichomonads undergoes adaptive changes *in vivo*, enabling parasites to avoid lysis by complement.

Iron is an important component of the host environment, and we have recently shown that iron regulates a variety of trichomonal properties. Most significant was the demonstration that iron regulated the gene expression of surface immunogens and adhesins.[7–9] We now demonstrate that iron in the growth medium directly influences the resistance by *T. vaginalis* to serum complement. This property illustrates an immune evasion strategy of the parasite, which undoubtedly contributes to microbial pathogenesis.

Results

Complement-mediated killing of T. vaginalis *and regulation of resistance to complement killing by iron*

Initially, a representative fresh *T. vaginalis* isolate, T048, was grown either in high- or low-iron medium and examined for susceptibility to complement-mediated lysis. Parasites were suspended in 10% normal human serum-Mg^{++}-EDTA (referred to as NHS, Materials and Methods) and monitored visually over a 5 min incubation period at 37°C. Phase and dark-field microscopy[10] revealed that low-iron parasites were readily lysed by $\geqslant$10% NHS. In contrast, organisms cultured in high-iron medium survived $>$1 h under the same conditions of $\geqslant$10% NHS. These initial observations were consistent with the reported sensitivity of *in vitro*-grown trichomonads to complement.[1]

Experiments monitoring the concentration-dependent lysis of parasites (Fig. 1A) reaffirmed the resistance of complement-mediated killing by high- versus low-iron *T. vaginalis* organisms. No lysis of high-iron trichomonads was apparent in up to 50% NHS. In contrast, 50% of parasites grown in normal trichomonal medium or in medium depleted of iron were lysed by $\leqslant$25% NHS. This is not surprising, since earlier work has shown that the normal trichomonal medium is deficient in iron for optimal metabolism,[11] expression of immunogens[9] and adhesins.[7,8] Trichomonads were never lysed under the same conditions in the absence of NHS or in the presence of identical concentrations of heat inactivated (HI)-NHS.

Only iron, not other divalent cations, when added to low-iron trichomonads rendered sensitive parasites resistant to complement (Fig. 1B). No toxicity of the cations to the parasites was observed, as previously shown[9] and as evidenced by the motility and viability of the organisms. Equally noteworthy was concentration-dependent effect of iron on resistance to complement. For example, trichomonads grown in normal medium (complement-sensitive) were transferred to medium differing in the concentration of iron or iron chelator (Fig. 2). Maximal lysis by complement was seen only in trichomonads grown in normal medium or under iron-limitation. Trichomonads grown at $\geqslant$100 μM ferrous iron were resistant. Collectively, these data show the specific, concentration-dependent nature of iron regulation of complement resistance.

Finally, it was important to test other fresh and long-term-grown isolates of *T. vaginalis*. As shown in Table 1, the iron-regulation of complement resistance was

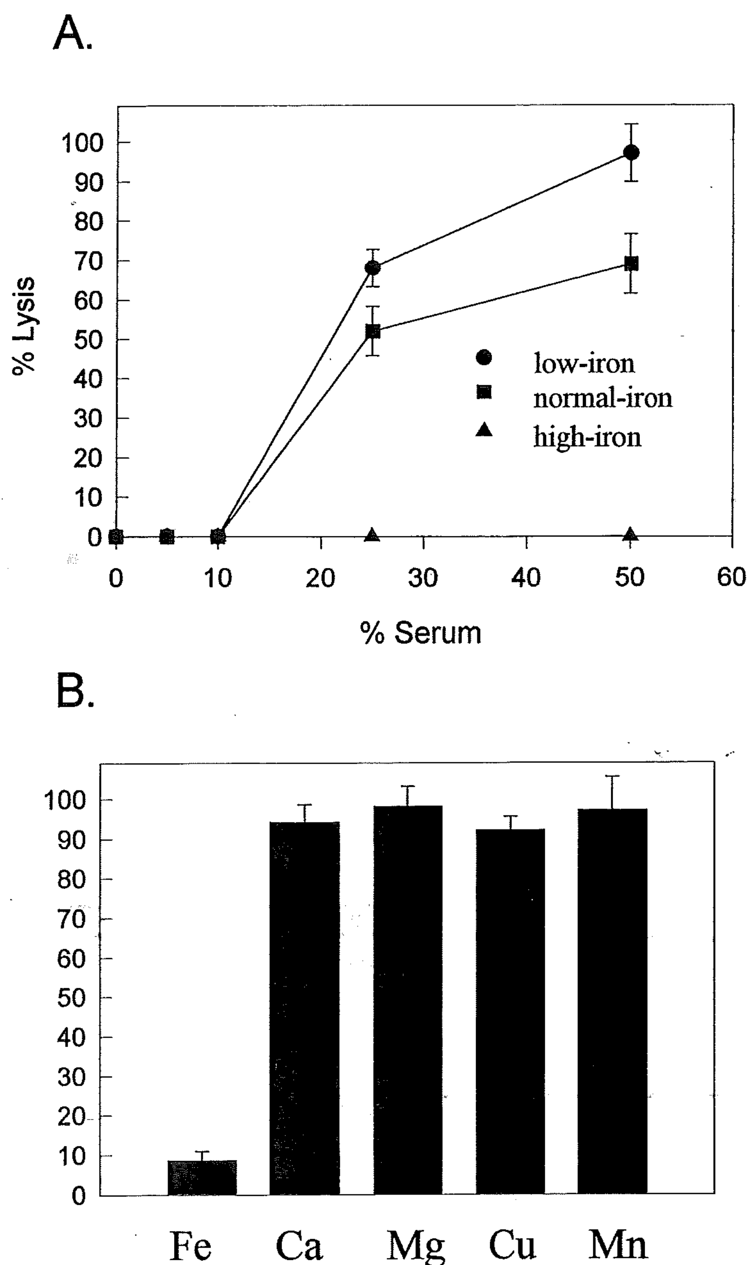

Fig. 1. Concentration-dependent lysis by serum complement of *T. vaginalis* isolate T048 grown in various media (part A) and specificity of ion-modulated expression of complement resistance (part B). (A) Percent of trichomonads lysed by complement were quantitated in flat bottomed, 96-well tissue culture trays containing 1×10^6 organisms in 100 μl medium with various concentration of NHS. Trichomonads were grown in normal medium of TYM-10% HI-HS (squares) or in normal medium supplemented with 2,2-DP (low-iron) (circles) or 250 μM iron (high-iron) (triangles). After incubation, cells excluding trypan blue were counted in a hemocytometer, and the percentage lysis was calculated (Materials and Methods). (B) The specificity of iron-regulated expression of complement resistance was determined by measuring the percent lysis of low-iron trichomonads after cultivation in normal TYM-10% HI-HS medium supplemented with 250 μM divalent cations. Abbreviations: Fe, ferrous ammonium sulfate; Ca, calcium chloride; Mg, magnesium chloride; Cu, cupric sulfate; Mn, manganese chloride.

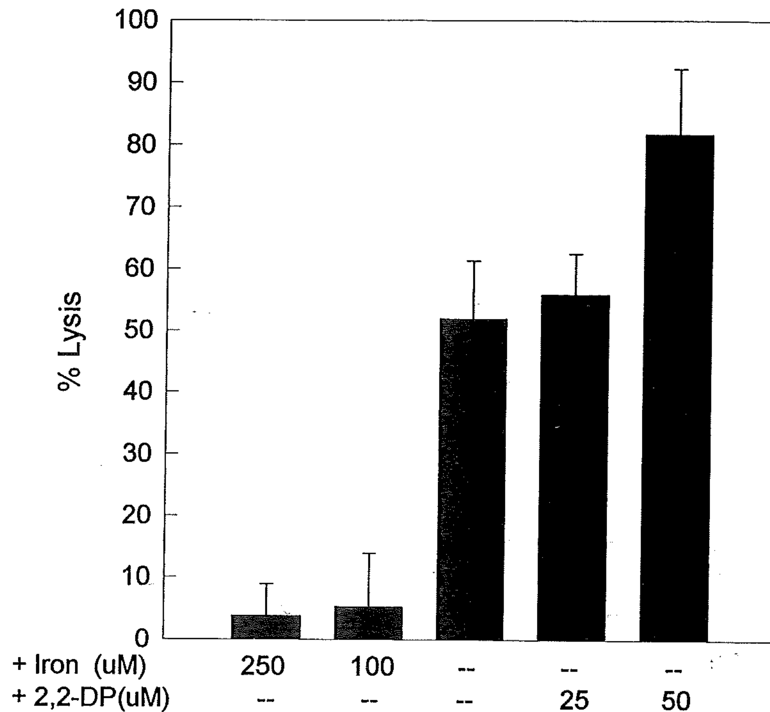

Fig. 2. Resistance to 20% NHS complement by *T. vaginalis* T048 is dependent on the concentration of iron. Trichomonads were grown in normal medium and then transferred to medium containing ferrous ammonium sulfate (high-iron) or 2,2-DP (low-iron). The percentage lysis of organisms grown in normal medium is shown in the middle bar. Parasites were grown for 12 h after transfer to the various media.

Table 1 Iron-induced serum resistance among five fresh *T. vaginalis* isolates and the representative long-term grown NYH 286 isolate

		Percent lysis[b]					
Growth medium[a]	Isolate:	T048	T056	T038	T015	T068	NYH 286
With iron		0	0	0	0	9.3	0
Without iron		50.3	48.5	33.7	60.2	49.5	49.5

[a]Trichomonads were added to medium with 100 μM 2,2-DP (low-iron) that was first acidified to pH 5.5. Parasites were also added to medium supplemented with 250 μM ferrous ammonium sulfate (high-iron) as described in Materials and methods.

[b]Trichomonads were grown to the mid-logarithmic phase of growth. Parasites were then harvested, washed with PBS and incubated with 20% NHS. Values for lysis were determined as described in the Materials and methods section and represent the mean of triplicate sample. The standard error for each sample was ⩽5% of percent lysis.

evident for representative isolates, including isolate NYH 286, the long-term-grown laboratory isolate.[12,13]

An in vivo iron source also renders trichomonads resistant to complement

Table 2 illustrates that parasites grown in low-iron medium supplemented with lactoferrin as a source of iron[8] had reduced lysis by complement as seen for trichomonads grown in iron-replete medium. Not surprisingly, transferrin, another

Table 2 Resistance levels to complement-mediated lysis of *T. vaginalis* grown in low-iron medium supplemented with different iron sources

Addition to low-iron medium[a]	Percent lysis[c]
None, control	96.0 ± 12.5
+iron[b]	0 ± 0
+lactoferrin[b]	6.2 ± 5.6
+transferrin	79.3 ± 8.6

[a,b] Trichomonads were grown in TYM-serum medium to mid-logarithmic phase of growth before harvesting and inoculation into low-iron medium containing 100 μM 2,2-dipyridal. Low-iron medium was also supplemented with either 250 μM ferrous ammonium sulfate, 1 mg/ml iron-saturated lactoferrin, or 1 mg/ml iron-saturated transferrin.
[c] Percent lysis was determined as described in Materials and methods. For this experiment 20% NHS was used in the complement lysis assay.

iron-binding protein that is not a source of iron for *T. vaginalis*[8,9,14] did not increase resistance to complement lysis.

Binding of C3 to high- and low-iron trichomonads

Measurement of iodinated C3 bound to live trichomonads (Fig. 3A) showed an 8-fold greater association of C3 with low-iron when compared to high-iron cells. However, it was intriguing that experiments performed in parallel using fixed organisms (Fig. 3B) demonstrated almost equal amounts of C3 binding for low- and high-iron organisms. Unlabeled C3 added to the reaction competed with iodinated C3 for binding, providing evidence of specific association between ^{125}I-labeled C3 and putative sites on *T. vaginalis* organisms.

We next performed a ligand-blot assay (Materials and methods) using trichomonads immobilized onto nitrocellulose to reaffirm the above observations. Results presented in Fig. 3C showed equal amount of C3 bound to parasites regardless of the iron status. That both high- and low-iron organisms, whether fixed or immobilized on nitrocellulose, were capable of binding similar amounts of C3 suggested the possibility that C3 might be removed from the surface of high-iron parasites.

T. vaginalis *cysteine proteinase removes surface-bound C3*

Iodinated C3 eluted from fixed trichomonads in the binding assay described above showed the same molecular form and amounts of C3 for high- and low-iron trichomonads. We, therefore, incubated parasites for various periods of time at 37°C with non-lytic amounts of NHS plus iodinated C3. In this way it might be possible to examine C3 on live *T. vaginalis* grown in high- versus low-iron medium. Figure 4A shows the iodinated α and β subunits and atypical degradation fragments generated by live high-iron trichomonads. No such degradation was seen when cells were coincubated with TLCK, a known inhibitor of the trichomonad cysteine proteinases.[15–18] Furthermore, no degradation of C3 was detected in identical coincubation experiments with low-iron parasites (Fig. 4B). These results suggested that resistance to complement of high-iron trichomonads was possibly mediated by proteinases.

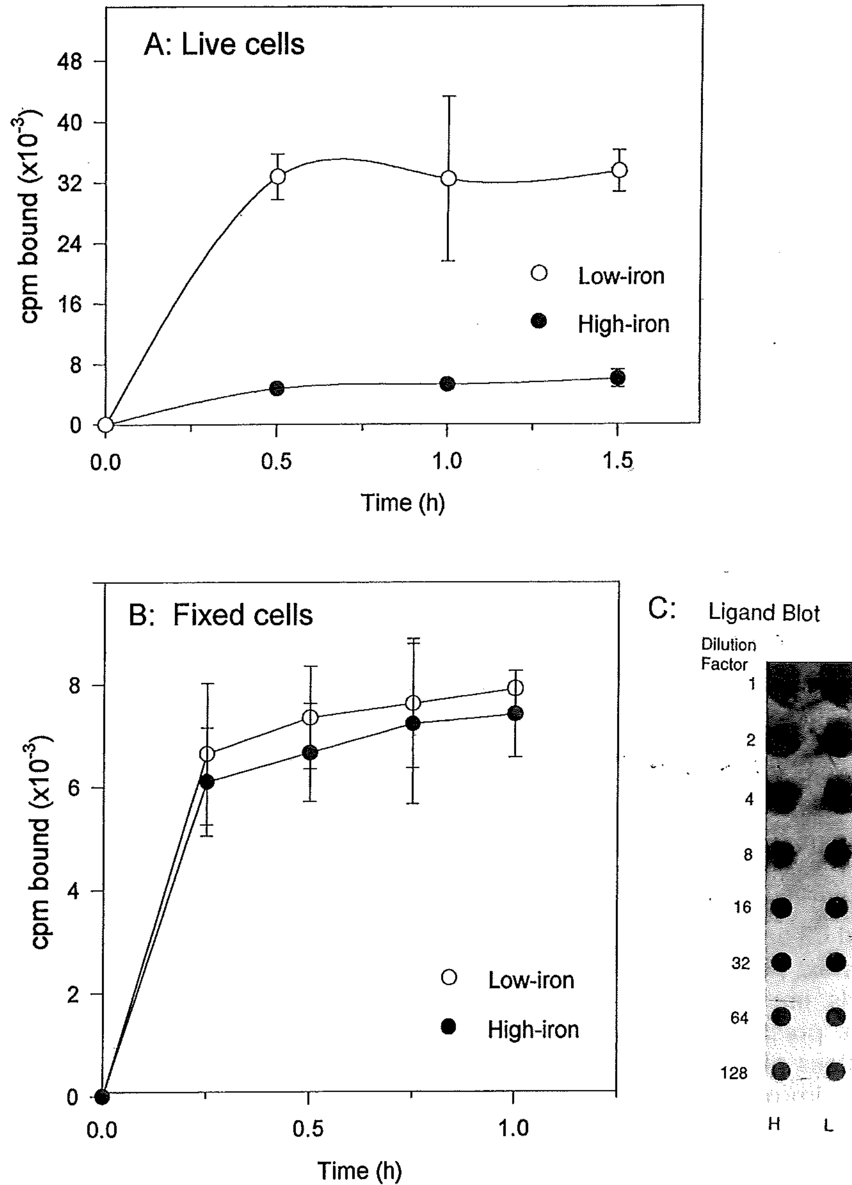

Fig. 3. Levels of C3 bound to live (A) and fixed (B) trichomonads grown in high- and low-iron medium incubated with iodinated C3 for different times. Details for measuring associated iodinated C3 after extensive washing of parasites is as described in Materials and Methods. As described in the text, sublethal amounts of NHS containing ^{125}I-labeled C3 were added to live trichomonads. Ligand blotting (C) was performed to verify that low- and high-iron organisms bound identical amounts of iodinated C3. In this case, parasites were immobilized onto nitrocellulose and blots incubated with C3, which was then detected with specific anti-C3 antibody.

Table 3 provides further evidence that high-iron trichomonads resistant to complement-mediated lysis can become sensitive if pretreated with inhibitors of cysteine proteinases.[16,18] Of particular interest was the effectiveness of E64 and leupeptin, which do not cross cell membranes. These results suggest the involvement of a surface proteinase in the resistance to complement-mediated lysis. As controls, proteinase inhibitors like phenylmethylsulfonyl fluoride (PMSF), antitrypsin, and

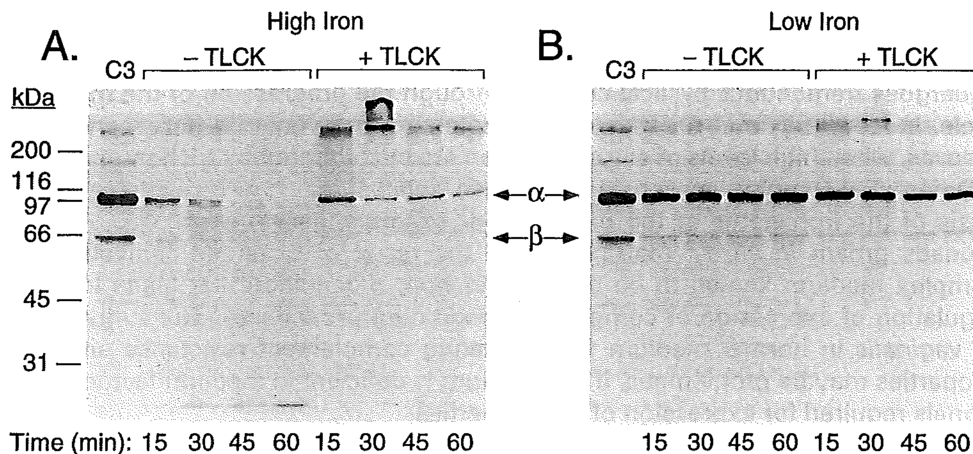

Fig. 4. Autoradiograms after SDS-PAGE showing the time-course binding and the molecular form of C3 associated with high- (A) and low-iron trichomonads (B). Parasites (1×10^7 per ml) were incubated in 2.5% NHS containing ^{125}I-C3 in the presence or absence of 400 μM TLCK. Cells were then removed at 15 min intervals, washed and solubilized in electrophoresis dissolving buffer for electrophoresis in 10% acrylamide gels and autoradiography. The bands labeled α and β refer to the C3 subunits, which were identified using purified C3 electrophoresed simultaneously (lane labeled C3). Numbers on the left refer to molecular size markers in kDa.

Table 3 Cysteine proteinase inhibitors abolish iron-induced resistance to complement-mediated lysis

Treatment[a]	Percent lysis[b]	
	Medium: High-iron	Low-iron
None	0	100
TLCK[c] (400 μM)	46.6±4.7	100
Leupeptin (0.5 mg/ml)	29.6±4.3	100
E64 (0.5 mg/ml)	56.8±8.4	100
Antitrypsin (1 mg/ml)	0	100
Aprotinin (1 mg/ml)	0	100
PMSF[c] (1 mM)	0	100

[a] Treatment consisted of addition of proteinase inhibitors to high- or low-iron grown cells 5 min prior to complement lysis assay.
[b] Parasites grown in low- and high-iron medium were incubated with 10% NHS for 1 h at 37°C. The values for percent lysis were calculated as before.
[c] TLCK, N-α-tosl-L-lysine chloromethyl ketone; PMSF, phenylmethylsulfonyl fluoride.

aprotinin, which do not inhibit trichomonad cysteine proteinases, were ineffective in abolishing iron-induced resistance.

Discussion

The data from this study reinforces the idea that complement component C3 binds to acceptor sites on the *T. vaginalis* surface and leads to death of the parasite through activation of the alternative complement pathway.[2] We also report that iron

influences resistance or susceptibility to complement-mediated lysis. Clearly, such a strategy would be beneficial for *T. vaginalis*, as it inhabits an ecological niche that undergoes tremendous cyclical changes through the progression of the menstrual cycle. Indeed, maximal resistance to complement may only be necessary during menses, when high levels of serum and iron are encountered by trichomonads.

Differential complement sensitivity of fresh isolates[1] may now be explained on the basis of the iron-status of the parasite. For example, serum sensitivity of trichomonads grown *in vitro*[1,2] may clearly be the result of extended cultivation in a complex medium known to be deficient in iron, a condition that leads to down-regulation of expression of complement-degrading proteinases. The cultivation of *T. vaginalis* in normal medium for evaluating complement resistance and other properties may be problematic, if the medium is deficient in medium factors and/or signals required for expression of the properties.

It has often been noted that symptoms of trichomoniasis are exacerbated shortly after menstruation,[20,21] the time at which high levels of complement are found in the vagina. Furthermore, it is known that active complement titers of menstrual blood vary among women.[22] It is possible that patients with diminished levels of menstrual blood complement will have parasites that survive lytic attack in contrast to trichomonads exposed to high levels of complement in menstrual blood. An increased parasitemia in these patients may lead to host pathology. In addition, during activation of the complement cascade, naturally-occurring peptides with biological function, such as the anaphylactic C3a molecule, are generated.[23] Whether proteolytic cleavage of C3 by trichomonad proteinases gives rise to similar bioactive peptides is unknown. Nonetheless, this possibility is intriguing and requires attention in the future.

The presence and the secretion of multiple proteinase activities in *T. vaginalis* has been appreciated for some time.[14–18,24] Proteinases contribute to nutrient acquisition through lysis of erythrocytes,[13] to parasite recognition and binding to host cells,[25] and to immune evasion by degradation of immunoglobulins.[26] We now further extend these earlier observations[17,24] and demonstrate a possible role for one or more trichomonad proteinases in evasion of the alternative complement pathway. Clearly, it is necessary to dissect the dual role that trichomonad proteinases may have in host pathology and survival of *T. vaginalis.* Although requiring experimental verification, the trichomonad proteinases may facilitate the transmission of other sexually transmitted diseases, because of impaired host defense mechanisms. Indeed, the coexistence of several STDs with trichomoniasis has been reported in the literature.[27–30]

The persistence of infection, despite the presence of lytic complement during menstruation,[21] is a hallmark of trichomoniasis. Without a doubt persistence is due to the ability of the parasite to respond to environmental signals, some of which allow for survival by circumventing the protective nature of host surveillance systems, such as that of complement. Overall, the complexity of the host-parasite interrelationship is illustrated, showing that *in vivo* signals, like iron, lead to adaptive responses that enable the parasite to survive in the hostile host environment.

Materials and methods

Parasite growth. *Trichomonas vaginalis* isolates used in this study have been described previously.[8,9,12] Trichomonads were grown in the complex Trypticase-yeast extract-maltose (TYM) medium supplemented with 10% heat-inactivated horse serum (HIHS).[19,31] Only mid to

late-logarithmic phase organisms were used for assays. As before,[8] low-iron medium was prepared by the addition to the growth medium of 2,2-dipyridal (2,2-DP) (Sigma Chemical Company, St. Louis, MO, U.S.A.) High-iron medium was made by the addition of ferrous ammoniumsulfate hexahydrate (Sigma) (250 mM final concentration from a 100-fold stock solution made in 50 mM sulfosalicylic acid).[11]

Sera and determination of total hemolytic complement and the complement assay. Venous blood was collected from normal volunteers with no history of trichomoniasis or other sexually transmitted diseases. After clotting at room-temperature (RT) for 2 h, the blood was centrifuged and serum aliquoted and stored at –70°C until use. The CH_{50} titer[32] of the pooled serum was 263 units/ml. Complement titers of sera were measured with the Diagnostic Comp Quik CH_{50} test kit (Sigma) according to specifications. Only sera without any detectable anti-trichomonad antibodies, as determined by various assays, including indirect immunofluorescence[10] and immunoblot,[8,33] were used. To inactivate the classical complement pathway while leaving the alternative complement pathway intact, normal human serum was treated with 10 mM ethylene glycol-bis-(β-aminoethyl ether-N,N,N′,N′ tetraacetic acid (EGTA; Sigma) and then supplemented with 1 mM $MgCl_2$ (referred to as just NHS unless otherwise indicated).[34] Experiments were always performed simultaneously with heat-inactivated sera.

Lysis of *T. vaginalis* was evaluated in flat-bottomed, 96-well tissue culture trays containing 1×10^6 organisms in 100 μl of TYM-medium supplemented with 150 μM $MgCl_2$. Fifty μl of TYM-medium containing 20% NHS was added and followed by addition of 150 μM EGTA[34] to each well. Plates were incubated at 37°C for 1 h. Increasing the time of incubation did not result in any significant increase in lysis.

After incubation, the lysis reaction was stopped by sequential addition of 100 μl of PBS-0.1% trypan blue and 50μl of 10% glutaraldehyde in PBS. Cells excluding trypan blue were counted in a hemocytometer, and the percentage lysis was calculated by the following equation: {(Number of viable parasites in control minus number of viable parasites in sample)/Number of viable parasites in control} × 100. All experiments were performed in triplicate and repeated at least three times.

Iodination of C3 and the C3 cell-binding Assay. Complement component C3 (Sigma) was radioiodinated by chloramine T iodination kit (ICN Biomedicals, Inc., Costa Mesa, CA, U.S.A.) as recommended by the manufacturer. Free radiosotope was removed by Sephadex G-25 chromatography.[14] Efficiency of labeling was determined by TCA precipitation.[35] The sp act of C3 ranged from 5×10^4 to 1×10^5 cpm/μg.

Binding of complement C3 to live trichomonads was measured during incubation in NHS, as described previously.[34] Briefly, 1×10^7 parasites in 1 ml TYM-10% HI-NHS or NHS and 10 μg of iodinated C3 were incubated for various times at 37°C. Parasites were then pelleted by centrifugation and washed three times with ice-cold PBS and transferred to another tube before determination of avidly bound C3 by scintillation counting. The specific binding was calculated as follows: (cpm pellet with NHS minus cpm pellet with HI-NHS). All experiments were done in triplicate and repeated at least three times. Cell binding assays with fixed cells were performed similarly. Washed cells were incubated with 0.5% glutaraldehyde in PBS for 1 h. Cells were then pelleted and washed three times with 10 mM glycine in PBS. Finally, cells were washed with PBS twice prior to measuring binding of Cs as described above.

For identification of bound C3, SDS-PAGE and autoradiography were performed as described previously.[10,33,36] Briefly, washed parasites of the C3 cell-binding assay were resuspended in electrophoresis dissolving buffer,[36] boiled for 3 min, and centrifuged to remove insoluble debris. After electrophoresis, gels were stained and dried before exposing to X-ray film. Molecular weight standards were always included (BioRad Laboratories, Richmond, CA, U.S.A.).

Ligand blotting. For ligand blotting, 10μl serial dilutions of washed trichomonads were spotted onto nitrocellulose (NC) and air dried. The NC was blocked for 1 h with non-fat dry milk (NFDM) in Tris-buffered saline (TBS, 20 mM Tris-HCl and 500 mM NaCl, pH 7.4) for 2 h at RT. Blots were then placed in a solution of PBS containing 10% NHS. After incubation at 37°C for 1 h, the blots were washed three times with PBS and incubated with rabbit anti-human C3 IgG diluted 1 : 100 (v/v) in NFDM-TBS-0.05% Tween 20. Blots were then incubated overnight at RT. Goat anti-rabbit IgG conjugated to horseradish peroxidase (Sigma) diluted 1 : 1000 in NFDM-TBS-0.05% Tween 20 was then added and incubated at RT for 6 h. The

blots were washed three times for 10 min with TBS-0.05% Tween 20 and developed with 4-chloro-1-napthol (Sigma) (2 mg/ml) prepared in TBS-20% methanol containing 0.015% H_2O_2.

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References

1. Demeš P, Gombošova A, Valent M, Jánoška A, Fabušová H, Petrenko M. Differential susceptibility of fresh *Trichomonas vaginalis* isolates to complement in menstrual blood and cervical mucus. Genitourin Med 1988b; 64: 176–9.
2. Gillin FD, Sher H. Activation of the alternative complement pathway by *Trichomonas vaginalis*. Infect Immun 1981; 34: 268–73.
3. Holbrook TW, Boakle RJ, Vesely J, Parker BW. *Trichomonas vaginalis*: alternative pathway activation of complement. Trans R Soc Trop Med Hyg 1982; 76: 473–5.
4. Reisenhover V. Uber die Beeinflussing von *Trichomonas vaginalis* durch verschiedene Sera. Arch Hyg Bakteriol 1963; 146: 628–35.
5. Weld JT, Kean BH. A factor in serum of human beings and animals that destroys *T. vaginalis*. Proc Soc Exp Biol Med 1958; 98: 494–6.
6. Demeš P, Gombošova A, Valent M, Fabušova H, Jánoška A. Fewer *Trichomonas vaginalis* organisms in vaginas of infected women during menstruation. Genitourin Med 1988a; 64: 22–4.
7. Alderete JF, O'Brien JL, Arroyo R *et al.* Cloning and molecular characterization of two genes encoding adhesion proteins involved in *Trichomonas vaginalis* cytoadherence. Molec Microbiol 1995; 17: 69–83.
8. Lehker MW, Arroyo R, Alderete JF. The regulation by iron of the synthesis of adhesins and cytoadherence levels in the protozoan *Trichomonas vaginalis*. J Exp Med 1991; 174: 311–8.
9. Lehker MW, Alderete JF. Iron regulates growth of *Trichomonas vaginalis* and the expression of immunogenic trichomonad proteins. Mol Microbiol 1992; 6: 123–32.
10. Alderete JF, Kasmala L, Metcalfe E, Garza GE. Phenotypic variation and diversity among *Trichomonas vaginalis* and correlation of phenotype with trichomonal virulence determinants. Infect Immun 1986; 53: 285–93.
11. Gorrell TE. Effect of culture medium iron content on the biochemical composition and metabolism of *Trichomonas vaginalis*. J Bacteriol 1985; 161: 1228–30.
12. Alderete JF. Antigen analysis of several pathogenic strains of *Trichomonas vaginalis*. Infect Immun 1983; 39: 1041–7.
13. Dailey DC, Chang TH, Alderete JF. Characterization of *Trichomonas vaginalis* haemolysis. Parasitol 1990; 101: 171–5.
14. Peterson KM, Alderete JF. Iron uptake and increased intracellular enzyme activity follow host lactoferrin binding by *Trichomonas vaginalis*. J Exp Med 1984; 160: 398–410.
15. Coombs GH, North MJ. An analysis of the proteinases of *Trichomonas vaginalis* by polyacrylamide gel electrophoresis. Parasitol 1983; 86: 1–6.
16. Lockwood BC, North MJ, Scott KI, Bremmer AF, Coombs GH. The use of a highly sensitive electrophoretic method to compare the proteinases of trichomonads. Mol Biochem Parasitol 1987; 24: 89–95.
17. Neale KA, Alderete JF. Analysis of the proteinases of representative *Trichomonas vaginalis* isolates. Infect Immun 1990; 58: 157–62.
18. North MJ, Robertson CD, Coombs GH. The specificity of trichomonad cysteine proteinases analyzed using fluorogenic substrates and specific inhibitors. Mol Biochem Parasit 1990; 39: 183–94.
19. Peterson KP, Alderete JF. Host plasma proteins on the surface of pathogenic *Trichomonas vaginalis*. Infect Immun 1982; 37: 755–62.
20. Krieger JN. Urologic aspects of trichomoniasis. Invest Urol 1981; 18: 411–7.
21. Rein MF, Chapel TA. Trichomoniasis, candidiasis and the minor venereal diseases. Clin Obstet Gynecol 1975; 18: 73–88.
22. Schumacher GFB, Kim MH, Hosseinian AH, Dupon C. Immunoglobulins, proteinase inhibitors, albumin, and lysozyme in human cervical mucus. Am J Obstet Gynecol 1977; 129: 629–36.
23. Joiner KA. Complement evasion by bacteria and parasites. Ann Rev Microbiol 1988; 42: 201–30.
24. Alderete JF, Newton E, Dennis C, Neale KA. The vagina of women infected with *Trichomonas vaginalis* has numerous proteinases and antibody to trichomonad proteinases. Genitourin Med 1991; 67: 469–74.
25. Arroyo R, Alderete JF. *Trichomonas vaginalis* proteinase activity is necessary for parasite cytoadherence. Infect Immun 1989; 57: 2991–6.

26. Provenzano D, Alderete JF. Analysis of human immunoglobulin-degrading cysteine proteinases of *Trichomonas vaginalis.* Infect Immun 1995; 63: 3388–95.
27. Hardy PH, Hardy JB, Nell EE, Graham DA, Spence MR, Rosenbaum RC. Prevalence of six sexually transmitted disease agents among pregnant inner-city adolescents and pregnancy outcome. Lancet 1984; ii: 333–7.
28. Laga M, Alary M, Nzila N, *et al.* Condom promotion, sexually transmitted diseases treatment, and declining incidence of HIV-1 infection in female Zairian sex workers. Lancet 1994; 344: 246–8.
29. Laga M, Manoka A, Kivuvu M, *et al.* Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: results from a cohort study. AIDS 1993; 7: 95–102.
30. Vuylsteke B, Bastos R, Barreto J, *et al.* High prevalence of sexually transmitted diseases in a rural area in Mosambique. Genitourin Med 1993; 69: 428–30.
31. Diamond LS. The establishment of various trichomonads of animals and man in axenic cultures. J Parasitol 1957; 43: 488–90.
32. Fine DP, Marney SR, Colley DG, Sergent JS, Des Prez RM. C3 shunt activation in human serum chelated with EGTA. J Immunol 1972; 109: 807–9.
33. Peterson KM, Alderete JF. Acquisition of α_1-antitrypsin by a pathogenic strain of *Trichomonas vaginalis.* Infect Immun 1983; 40: 640–6.
34. Fuhrman SA, Joiner KA. *Toxoplasma gondii*: Mechanism of resistance to complement-mediated killing. J Immunol 1989; 142: 940–7.
35. Garvey JS, Cremer NE, Sussdorf DH. ^{125}I or ^{131}I-labeled proteins. In: Campbell, D. H., Ed. Methods in Immunology. Reading, MA: Benjamin, W.A., Inc., 1977: 171–82.
36. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680–5.