IRON UPTAKE AND INCREASED INTRACELLULAR ENZYME ACTIVITY FOLLOW HOST LACTOFERRIN BINDING BY *TRICHOMONAS VAGINALIS* RECEPTORS

BY KENNETH M. PETERSON AND JOHN F. ALDERETE

From the Department of Microbiology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284

Trichomonal vaginitis caused by *Trichomonas vaginalis* represents the most frequently acquired protozoan infestation (1–4). Despite the emergence of trichomoniasis as a major sexually transmitted disease, only limited information is available concerning *T. vaginalis* mechanisms of disease pathogenesis. Determinants of pathogenicity have not been identified, although contact-dependent cytolytic events mediating destruction of host cells and trichomonal hemolytic activity have been reported (2, 5, 6). The parasite molecules responsible for these observations, however, are still unknown.

Because acquisition of host substances is important to the pathogenic mechanisms of microorganisms (7–14), we have attempted to define the molecular interactions of host macromolecules with *T. vaginalis* (15–17). Our approach has involved a step-wise examination of plasma protein acquisition, identification of host proteins enriched onto trichomonad surfaces, and elucidation of biological properties conferred upon *T. vaginalis* through binding and uptake of certain host molecules (16, 17).

In a recent study (17) we reported the avid association of several host plasma proteins, including an iron-binding protein, with the surface of *T. vaginalis*. Transferrin and lactoferrin are two proteins, with high binding affinities for ferric ions (18, 19), which are involved in iron sequestration within host tissues. Transferrin is found predominantly in plasma, while lactoferrin is produced by acinar cells of mucosal surfaces and is found in milk, semen, tears, cervical secretions, polymorphonuclear leukocytic granules, and pus (20–22). Because acquisition of host iron through specific mechanisms may be important to *T. vaginalis* (23–25), we decided to characterize further the interaction of these two iron-binding proteins with *T. vaginalis*. These data show that iron accumulation and increased intracellular enzyme activity are associated with the binding of human lactoferrin by specific receptors on *T. vaginalis*.

Materials and Methods

Growth of Organisms. *T. vaginalis* strains NYH 286, 272, ATCC 30001, 30236, RU 375, and IR 78 were kindly provided by Miklós Müller, The Rockefeller University, New

J. F. A. was supported by Public Health Service grants AI-18768 and AI-19142 from the National Institutes of Allergy and Infectious Diseases of The National Institutes of Health, and was the recipient of Research Career Development Award K04 AI00584 from the National Institutes of Health. Correspondence should be addressed to Dr. John F. Alderete.

 J. EXP. MED. © The Rockefeller University Press · 0022-1007/84/08/0398/13 $1.00
Volume 160 August 1984 398–410

York. Strains JHHR and JHHW were provided by Michael Spence, Johns Hopkins. These strains were passaged daily in Diamond's trypticase-yeast extract-maltose (TYM)[1] medium (26) supplemented with 10% normal human plasma. Only late log stage parasites were used in these studies (15). Where indicated, low density lipoproteins at levels equivalent to those found in plasma were added to the medium. The growth properties of *T. vaginalis* in low density lipoprotein–supplemented medium have been recently described (17).

Lactoferrin and Transferrin Preparation. Human milk lactoferrin was purified by heparin-agarose chromatography (27). Briefly, 300 ml of pooled human milk donated by La Leche League (San Antonio, TX) was thawed and the cream removed by centrifugation at 10,000 *g* for 20 min. The skim milk was adjusted to pH 4.7 with 1 N HCl and incubated at 40°C for 30 min followed by centrifugation at 10,000 *g* for 30 min. The whey was removed and dialyzed against 2 changes of 0.05 M NaCl in 0.005 M barbituric acid (Sigma Chemical Co., St. Louis, MO), pH 7.4. This material was then loaded onto a 1 × 45 cm heparin-agarose column equilibrated with the barbital buffer described above. After washing the column with starting buffer, a 500-ml linear gradient from 0.05 M to 1.0 M NaCl in starting buffer was applied at 15 ml/h. The peak corresponding to lactoferrin was pooled and dialyzed against PBS. Lactoferrin was >99% pure as determined by 7.5% polyacrylamide gel electrophoresis and staining. Serum transferrin (Sigma) was also evaluated for purity. Protein solutions of lactoferrin and transferrin were prepared in phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 4.6 mM Na_2HPO_4, and 1.5 mM KH_2PO_4), pH 7.4, and dialyzed overnight against two changes of 0.1 M citrate acetate buffer (pH 4.0) followed by one change of distilled water and two changes of a 40 mM Trizma base (Sigma)–20 mM sodium bicarbonate buffer (pH 7.4) (28). Saturation of transferrin and lactoferrin with iron was achieved by addition of 30 μg of $FeCl_3$ to 5 mg of protein in the Tris-bicarbonate buffer. Unbound iron was removed by dialysis against PBS.

Transferrin and lactoferrin were radioiodinated by lactoperoxidase as described previously (16, 17, 29) using 1.0 mCi of $Na^{125}I$ (Amersham Corp., Arlington Heights, IL). Unbound radioactivity was removed using a 1 × 15 cm Sephadex G25 (Pharmacia, Piscataway, NJ) column. Efficiency of labeling was determined by trichloroacetic acid precipitation (29).

For studies examining the uptake of ferric iron, $^{59}FeCl_3$ (Amersham) was added to transferrin and lactoferrin as above in the Tris-bicarbonate buffer for 30 min at 22°C. Unincorporated iron was removed by chromatography in a 1 × 10 cm Sephadex G25 column followed by overnight dialysis against two changes of PBS. These radiolabeled preparations were ~50% saturated with $^{59}Fe^{3+}$ as determined by incorporated radioactivity.

Cell Binding Assays. Live, motile parasites were washed twice in PBS to remove loosely bound medium components (17) and resuspended in PBS to a density of 1 × 10^8 cells/ml. 100-μl aliquots of this parasite suspension were then added to 1.5 ml siliconized conical polypropylene microfuge tubes pretreated with 1% horse serum (12). Different amounts of protein preparations were added to the parasites with a final volume of 500 μl. Incubation was at both 4°C and 37°C for various times with occasional gentle shaking. Trichomonads were separated from unbound material by gentle centrifugation (17), followed by two washings in ice cold PBS. Finally, pelleted organisms were resuspended and transferred to another tube before determination of avidly bound cpm. Competition experiments were performed similarly with increasing amounts of unlabeled proteins mixed with radiolabeled proteins before addition. Ratios of unlabeled to iodinated proteins are indicated in the legend of Fig. 4, illustrating the competition experiment.

Pyruvate/Ferredoxin Oxidoreductase Activity. *T. vaginalis* enzyme activity was monitored spectrophotometrically at 30°C, utilizing methylviologen as an artificial electron acceptor (23, 24). Briefly, an assay mixture containing 180 mM Na_2PO_4 and 120 mM $NaHPO_4$,

[1] *Abbreviations used in this paper:* Kd, kilodalton; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonylfluoride; RIP, radioimmunoprecipitation; SDS, sodium dodecyl sulfate; Staph A, protein A–bearing *Staphylococcus aureus*; TYM, trypticase-yeast extract-maltose; Z(3–12), Zwittergent 3 12.

pH 7.0, 16 mM methylviologen, 0.04 mM coenzyme A, 570 mM 2-mercaptoethanol, and 0.1% Triton X-100 was prepared. The solution was placed in a rubber-stoppered quartz glass cuvette and flushed with deoxygenated N_2. Parasites grown under different conditions were washed twice with anaerobic phosphate buffer (180 mM Na_2HPO_4 and 120 mM $NaHPO_4$, pH 7.0) and introduced into the assay mixture. After additional N_2 flushing, 4 mM (final concentration) pyruvate was added. The absorbance at 600 mM was monitored using a Beckman model 25 spectrophotometer. Results are expressed as nanomoles of reduced methylviologen formed per minute per 10^7 cells (24).

SDS-PAGE and Fluorography. One-dimensional SDS-PAGE was performed according to Laemmli (30), using 1.5-mm slab gels consisting of a 3% stacking and 7.5% separating gel (15). After electrophoresis, gels were fixed and dried for exposure to x-ray film (17) or processed for fluorography (15). Molecular weight standards were purchased from Bio-Rad Labs (Richmond, CA).

Affinity Purification of Trichomonad Lactoferrin-binding Proteins. A total of 5×10^9 ^{35}S-labeled (15) or surface radioiodinated trichomonads (31) were resuspended in 10 ml PBS containing 1% (wt/vol) Zwittergent 3-12 [Z(3–12)] (Calbiochem-Behring Corp., La Jolla, CA) detergent with 0.01% (wt/vol) phenylmethylsulfonylfluoride (PMSF). Trichomonads were solubilized by gentle homogenization and insoluble material removed by centrifugation at 40,000 *g* in a Sorvall RC-5B refrigerated superspeed centrifuge for 60 min. The soluble trichomonal extract was then further diluted in PBS-0.01% PMSF to give a final Z(3–12) concentration of 0.1%. Chromatography was performed on a 1×5 cm lactoferrin-Sepharose (32) affinity column followed by washing with PBS-0.01% PMSF. The adherent material was eluted with 3 M potassium thiocyanate, pH 7.2, and dialyzed against PBS-PMSF before SDS-PAGE-autoradiography.

Immunoprecipitation of Lactoferrin-trichomonad Receptor Complexes. A volume of 5×10^7 solubilized, ^{35}S-labeled parasites prepared as described above was aliquoted into individual microfuge tubes. 30 μg of human lactoferrin or serum transferrin was then added to the samples. and incubated overnight at 4°C with constant gentle rocking. 20 μl of specific antiserum to lactoferrin or transferrin was then added for an additional 6 h followed by a 2-h incubation with washed formalin-fixed protein A–bearing *Staphylococcus aureus* (Staph A) (31). Receptor-ligand-antibody complexes were then selectively removed by pelleting the Staph A. The Staph A were then washed twice with PBS-0.01% Z(3–12) before solubilization of receptor-ligand complexes (31) for SDS-PAGE-fluorography. High-titered monospecific antiserum against lactoferrin and transferrin were generated in New Zealand White rabbits using standard immunizing regimens (15). Prebleed serum served as a control for these assays.

Results

Time Course and Temperature-dependent Lactoferrin Binding. The time course binding of ^{125}I-labeled human lactoferrin to *T. vaginalis* at both 4°C and 37°C is shown in Fig. 1. The rates of acquisition and the amount of lactoferrin bound were similar at both temperatures, although ~20% more radioactivity was associated with trichomonads at the elevated temperature. Steady state binding was demonstrated at 37°C for up to 120 min. These kinetic studies implicate the presence of specific membrane sites for trichomonal acquisition of this host substance. The increased binding observed at 37°C may be due to nonspecific interactions or may reflect the increased fluidity of the parasite membrane at 37°C. Alternatively, additional synthesis or expression of membrane receptors at this temperature may result in enhanced specific acquisition.

Placement of organisms in PBS at 4°C or 37°C after maximal binding of iodinated lactoferrin (40 min) resulted in release of lactoferrin into the supernatant (Table I). ~75% of bound material dissociated from intact organisms after an additional 30-min incubation and was recovered in supernatant. No alteration

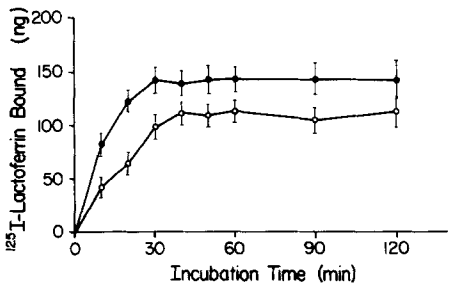

FIGURE 1. Representative time course binding of ^{125}I-labeled human lactoferrin to *T. vaginalis*. Organisms were suspended at a density of 1×10^7 parasites in a 0.5 ml final volume and incubated with 200 μg of ^{125}I-labeled-lactoferrin at 4°C (○) or 37°C (●). The amount of cell associated radioactivity was determined as described in Materials and Methods. Results of duplicate determinations of a single experiment are shown.

TABLE I

Release of ^{125}I-Lactoferrin from T. vaginalis Surfaces

Time of incubation	Incubation temperature	cpm associated with* *T. vaginalis* (% remaining)
0	4°C	36,000 (100)
5		31,000 (86)
15		18,000 (51)
30		9,000 (25)
45		8,000 (23)
0	37°C	46,000 (100)
5		38,000 (83)
15		16,000 (45)
30		10,000 (30)
45		9,000 (26)

* 1×10^7 organisms in 0.5 ml PBS were incubated with 100 μg ^{125}I-labeled lactoferrin (specific activity, 6×10^5 cpm/μg protein) at 4°C and 37°C for 40 min. After incubation at the specified temperatures, trichomonads were washed twice with PBS and resuspended in 0.5 ml PBS and transferred to another tube for the indicated times. Radioactivity was measured after pelleting of organisms as described in Materials and Methods. Each point represents the mean of three samples from a representative experiment. The standard deviation for each point never exceeded 10% of the mean.

in the electrophoretic mobility of either released or trichomonad-associated ^{125}I-labeled lactoferrin was observed (data not shown), indicating the absence of parasite degradation of lactoferrin.

Concentration-dependent Acquisition of ^{125}I-labeled Lactoferrin. Saturation binding of lactoferrin was achieved after incubation of *T. vaginalis* with increasing concentrations of iodinated ligand for 40 min at 4°C, (Fig. 2). The binding kinetics of the labeled lactoferrin used in these assays was not affected by the iron content of this protein. Low-level association of transferrin with *T. vaginalis* was observed under similar experimental conditions. The overall extent of transferrin binding to the parasites never exceeded 10% of that observed for lactoferrin. Scatchard analysis (33) of the data for lactoferrin binding are shown

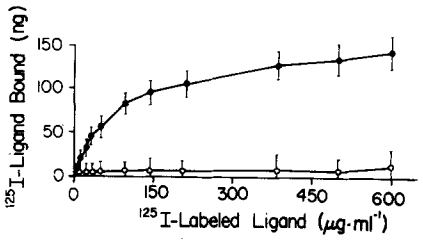

FIGURE 2. Concentration-dependent binding of ^{125}I-labeled human lactoferrin (●) and transferrin (○) to *T. vaginalis*. Cells were suspended at a density of 1×10^7 organisms in 0.5 ml PBS and incubated with varying concentrations of ligand for 40 min at 4°C. Parasite bound cpm was determined as described. Each data point represents the mean value of duplicate samples in three separate experiments.

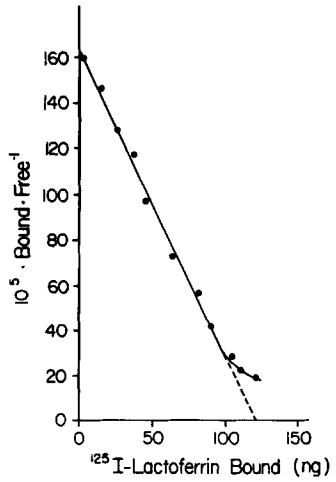

FIGURE 3. Scatchard plot analysis of data as presented in Fig. 2 which demonstrated saturation kinetics of ^{125}I-labeled human lactoferrin binding to *T. vaginalis*.

in Fig. 3 and provided evidence for a single class of lactoferrin receptors with an apparent K_d of 1.0 μM. Low affinity (nonspecific) interactions were also evident at very high lactoferrin concentrations. The average number of trichomonad lactoferrin receptors was estimated to be 9×10^4 per cell.

Lactoferrin Receptor Specificity. Stoichiometric competition for the lactoferrin receptor was observed when unlabeled ligand was added to trichomonads incubated with ^{125}I-labeled lactoferrin (Fig. 4). Unlabeled transferrin did not compete for ^{125}I-lactoferrin binding, demonstrating the specificity of the trichomonad receptor for lactoferrin.

Affinity-purified Trichomonad-lactoferrin Receptors. The identification of the membrane components involved in lactoferrin binding was attempted using affinity chromatography. Electrophoretic analysis of material eluted from lactoferrin covalently coupled to cyanogen bromide–activated Sepharose is presented in Fig. 5. Lane 1*A* shows ^{35}S-labeled proteins, with molecular weights (mol wts) of 178 Kd and 75 Kd, eluted from the lactoferrin column with 3 M potassium ferricyanide. Proteins with similar mol wts were identified when an extract of iodinated cells was used (lane 1*B*), confirming the surface origin of these

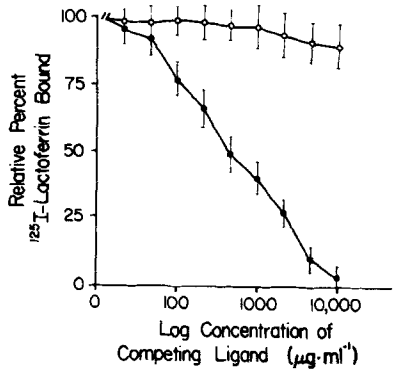

FIGURE 4. Specificity of acquisition of lactoferrin by *T. vaginalis*. A 1-ml suspension of 1×10^7 trichomonads were simultaneously incubated for 40 min at 4°C with 300 μg radioiodinated lactoferrin and varied amounts of unlabeled lactoferrin (●) or transferrin (○). 100 μg of unlabeled lactoferrin represents a ratio of 1:3 unlabeled to labeled protein. The amount of ^{125}I-labeled lactoferrin bound to trichomonads in the absence or presence of competing ligand was determined as described in Materials and Methods. Each data point represents the mean value of duplicate samples from two separate experiments.

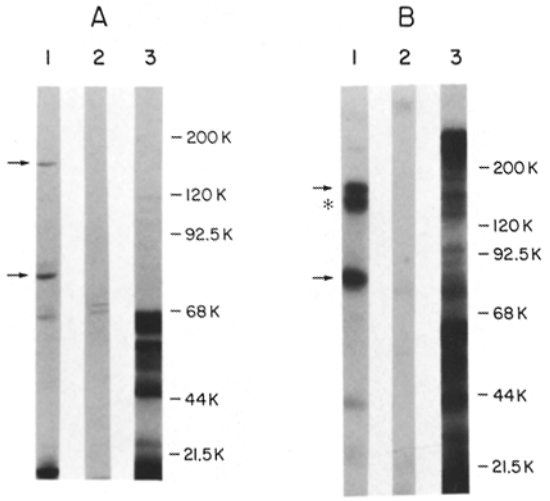

FIGURE 5. SDS-PAGE-radioautography of ^{35}S-intrinsically (*A*) and ^{125}I-extrinsically (*B*) radiolabeled *T. vaginalis* proteins purified by lactoferrin-Sepharose affinity chromatography. The trichomonad proteins selectively bound to the lactoferrin column are shown in lane *1*. The absence of parasite proteins binding to transferrin-Sepharose as a control is shown in lane *2*. Lane *3* shows the protein gel patterns of radiolabeled *T. vaginalis* Zwittergent extracts. Molecular weight markers: myosin, 200 K; beta-galactosidase, 120 K; phosphorylase *β*, 92.5 K; bovine serum albumin, 68 K; ovalbumin, 44 K; and soybean trypsin inhibitor 21.5 K (K = 10^3 daltons). Arrows denote lactoferrin-binding proteins.

lactoferrin-binding proteins. The absence of reducing agent in the electrophoresis dissolving buffer yielded identical gel patterns. Lanes 2*A* and 2*B* illustrate the inability of ^{35}S-labeled or iodinated parasite proteins, respectively, to interact with transferrin-Sepharose under identical conditions. Lanes 3*A* and 3*B* represent patterns of radiolabeled proteins in the detergent extracts subjected to chromatography and show the enrichment on lactoferrin-Sepharose for specific

parasite proteins. While the appearance of another (third) protein with a slightly lower mol wt by affinity chromatography of iodinated *T. vaginalis* extracts (Fig. 5, asterisk) cannot be explained at present, the absence of the doublet band using the alternative method (Fig. 6) suggests it may represent nonspecific material.

Immunoprecipitation of Lactoferrin-receptor Complexes. Another approach for identification of trichomonad components that bind human lactoferrin involved a radioimmunoprecipitation-type assay (34) using monospecific antisera to lactoferrin. A detergent extract of intrinsically labeled *T. vaginalis* was incubated with lactoferrin and anti-lactoferrin antibodies. Immunoprecipitated trichomonad proteins that bind lactoferrin are shown in Fig. 6 (lane *1*). These proteins possessed mol wts similar to those purified by affinity chromatography (Fig. 5). Increasing the concentration of lactoferrin in the reaction mixture resulted in increased immunoprecipitated radioactivity (data not shown). Addition of albumin or transferrin to the reaction mixture containing anti-lactoferrin serum did not result in precipitation of radiolabeled proteins (data not shown). Normal rabbit serum did not precipitate proteins under identical conditions (data not shown). The inability of transferrin and anti-transferrin antibodies to immunoprecipitate trichomonad proteins is shown in lane *2*. Finally, use of a detergent extract of iodinated *T. vaginalis* yielded equivalent results, reinforcing the surface orientation of the parasite lactoferrin receptors (data not shown).

T. vaginalis Uptake of Iron from Lactoferrin. We next examined the ability of these parasites to remove $^{59}Fe^{3+}$ from human ^{59}Fe-lactoferrin. As can be seen in

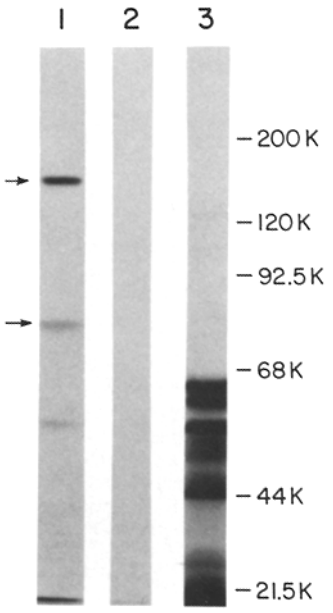

FIGURE 6. SDS-PAGE-radioautography following radioimmunoprecipitation of ^{35}S-labeled *T. vaginalis* lactoferrin-binding proteins. Specific proteins complexed with lactoferrin are shown in lane *1*. Lane *2* represents absence of *T. vaginalis* transferrin-binding proteins in a transferrin-antitransferrin mixture. Lane *3* shows the gel pattern of the Zwittergent extract of ^{35}S-labeled *T. vaginalis* used in this assay. Molecular weight markers are as indicated in the legend of Fig. 6. Arrows denote lactoferrin-binding proteins.

Fig. 7, *T. vaginalis* rapidly and efficiently removed ^{59}Fe from lactoferrin. On a molar basis, *T. vaginalis* acquired approximately 120-fold more iron (60 pM) at 37°C as compared with the steady-state concentration (0.5 pM) of iron bound to the parasite surface at 4°C. These data demonstrate parasite accumulation of iron in excess to the amounts of lactoferrin acquired by *T. vaginalis* at each time point at 37°C. Consistent with our earlier observation (Fig. 2), *T. vaginalis* was unable to accumulate radioactive iron from transferrin (data not shown).

Attempts by us to show the intracellular disposition of iron through subcellular fractionation of *T. vaginalis* were unsuccessful. Therefore, pyruvate/ferredoxin oxidoreductase, an enzyme involved in trichomonal energy metabolism (23) whose activity may be dependent upon intracellular iron levels, was monitored as a marker for iron uptake. As can be seen in Table II, addition of ferrous sulfate (a source of free iron) to TYM-medium increased this enzyme's activity more than sixfold. A similar enhancement of activity was observed with lactoferrin-supplemented growth medium. Transferrin, apolactoferrin and apotransferrin, however, did not stimulate pyruvate/ferredoxin oxidoreductase activity above control values. Finally, enzyme activity remained high upon addition to medium of apotransferrin in the presence of iron-lactoferrin. These data indicate that iron uptake results from specific acquisition of human lactoferrin by *T. vaginalis*.

Comparative Lactoferrin Binding among T. vaginalis Isolates. Potential variability in lactoferrin binding among several *T. vaginalis* isolates was assessed in the cell binding assay. Excess levels of ^{125}I-lactoferrin (500 μg/ml) were incubated with 1×10^7 parasites for 40 min at 4°C. Lactoferrin binding (Table III) varied no more than 30%, indicating little or no differences in receptor number among various trichomonal isolates.

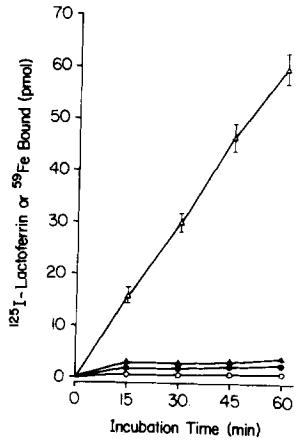

FIGURE 7. Time course iron (^{59}Fe) accumulation by *T. vaginalis*. Trichomonads (1×10^7) were resuspended in 0.5 ml PBS and incubated with 200 μg of ^{59}Fe-labeled lactoferrin (Δ, ○) or ^{125}I-labeled lactoferrin (▲, ●) at 37°C (▲, Δ) and 4°C (●, ○) for the indicated time points. The amount of radiolabel associated with *T. vaginalis* was determined as before. Each data point represents the mean value of duplicate samples from three experiments.

TABLE II

Pyruvate/Ferredoxin Oxidoreductase Activity

Medium* supplement	Activity (nmol $min^{-1}mg^{-1}$ of protein)‡
Control—no addition	260 ± 50¶
Ferrous sulfate§	1,670 ± 290
Lactoferrin‖	1,830 ± 330
Apolactoferrin‡	260 ± 60
Transferrin	230 ± 80
Apotransferrin‡	270 ± 90
Lactoferrin + apotransferrin	1,780 ± 350

* 1×10^7 *T. vaginalis* organisms were grown in TYM medium supplemented with human low density lipoproteins at a concentration equivalent to that found in 10% plasma (17).

‡ As described in Materials and Methods.

§ 25 mM ferrous ammonium sulfate was prepared in 50 mM sulfosalicylic acid. Final iron concentration in medium was 1.0 μg/ml.

‖ 100 μg/ml final concentration of 30% iron saturated lactoferrin or transferrin was used in the incubation medium as described above.

¶ Mean values of duplicate samples from three independent assays.

TABLE III

Representative Lactoferrin Binding among T. vaginalis Strains

Strain designation*	Lactoferrin avidly bound (ng)‡
NYU 286	120§
NYU 272	110
NYU IR 78	100
ATCC 30001	130
ATCC 30236	140
RU 375	140
JHHR	120
JHHW	140

* See Materials and Methods.

‡ 1×10^7 *T. vaginalis* organisms were grown in TYM-medium supplemented with human low density lipoproteins to a density of ~1×10^6 organisms/ml. Parasites were washed and then resuspended in 500 μg/ml of ^{125}I-lactoferrin (400 cpm/ng protein) for 40 min at 4°C. Cells were then washed and radioactivity bound determined as described in Materials and Methods.

§ Mean value of triplicate samples. Deviations never exceeded 20% of reported values.

Discussion

Successful parasitism by some pathogenic microorganisms is related to the uptake of host substances such as iron. Host iron is usually bound by specific molecules such as transferrin and lactoferrin, glycoproteins with high binding affinities for iron found in serum or vaginal mucosa, respectively. The acquisition of these host proteins by *T. vaginalis* through receptor-mediated processes might represent mechanisms by which these pathogenic human trichomonads acquire iron, and enhance their survival within the host environment.

In this report the data indicate that *T. vaginalis* possesses specific receptors for human lactoferrin. Each cell possesses ~9×10^4 receptors with an apparent K_d

estimated to be 1.0 μM (Fig. 3). The rate of association of the ligand with these parasites is equally rapid whether binding takes place at 4°C or 37°C (Fig. 1), supporting the idea of receptor-mediated acquisition of this host substance. Transferrin, an iron transport protein found in plasma, does not bind to *T. vaginalis* with high affinity (Fig. 2) and fails to compete for the binding of ^{125}I-labeled lactoferrin to intact trichomonads (Fig. 4). The ability of *T. vaginalis* to bind lactoferrin more effectively than plasma-derived transferrin may be a significant factor in the non-invasive nature of this parasite.

Chromatography of a Zwittergent extract of intrinsically or extrinsically labeled *T. vaginalis* on a lactoferrin-Sepharose affinity matrix resulted in purification of two trichomonad proteins (Fig. 5). Similar results were obtained using a different experimental approach involving a radioimmunoprecipitation-type assay system (34). An extract of ^{35}S-labeled parasite proteins in both experiments implicated two polypeptides (178 Kd and 75 Kd) as the putative lactoferrin receptors (Figs. 5 and 6).

The binding of lactoferrin to *T. vaginalis* results in intracellular iron accumulation (Fig. 7). These data did not allow us to discriminate whether iron is removed from lactoferrin at the parasite surface or whether lactoferrin is internalized before iron uptake. The release of lactoferrin after binding to the trichomonad receptor may indicate that the former sequence of events takes place allowing for a continuous turnover of exogenous lactoferrin.

An increase in the activity of pyruvate/ferredoxin oxidoreductase, an intracellular enzyme important for electron transport in this facultative anaerobic protozoan, resulted from *T. vaginalis* acquisition of human lactoferrin. These results are consistent with the importance of iron-containing proteins to the metabolic processes of *T. vaginalis* cells (23). These data and a report demonstrating the increased virulence of trichomonads following iron injection in mice (25) suggest that the trichomonad receptors for human lactoferrin contribute to the virulence of this parasite.

These data and previous work (15–17) have made evident the usefulness of host protein acquisition by *T. vaginalis* through processes that represent a clear departure from nonspecific coating of the parasite. It is possible that receptors binding biologically important host macromolecules such as lactoferrin, lipoproteins, α_1-antitrypsin, and other host molecules (15–17) contribute to the parasitic capabilities of this protozoan. We hope this information and our approaches will allow us to better understand the biology of this host-parasite relationship.

Summary

Lactoferrin acquisition and iron uptake by pathogenic *Trichomonas vaginalis* was examined. Saturation binding kinetics were obtained for trichomonads using increasing amounts of radioiodinated lactoferrin, while no significant binding by transferrin under similar conditions was achieved. Only unlabeled lactoferrin successfully and stoichiometrically competed with ^{125}I-labeled lactoferrin binding. Time course studies showed maximal lactoferrin binding by 30 min at 37°C. Data suggest no internalization of bound lactoferrin. The accumulation of radioactivity in supernatants after incubation of *T. vaginalis* with ^{125}I-labeled lactoferrin and washing in PBS suggested the presence of low affinity sites for

this host macromolecule. Scatchard analysis indicated the presence of 90,000 receptors per trichomonad with an apparent K_d of 1.0 μM. Two trichomonad lactoferrin binding proteins were identified by affinity chromatography and immunoprecipitation of receptor-ligand complexes. A 30-fold accumulation of iron was achieved using ^{59}Fe-lactoferrin when compared to the steady state concentration of bound lactoferrin. The activity of pyruvate/ferrodoxin oxidoreductase, an enzyme involved in trichomonal energy metabolism, increased more than sixfold following exposure of the parasites to lactoferrin, demonstrating a biologic response to the receptor-mediated binding of lactoferrin. These data suggest that *T. vaginalis* possesses specific receptors for biologically relevant host proteins and that these receptors contribute to the metabolic processes of the parasites.

We thank Miklós Müller and Tom Gorrell of The Rockefeller University for their gracious advice and assistance rendered during the oxidoreductase enzyme experiments. We gratefully acknowledge Barry Muhoberac and Charlie Gauntt for allowing us to use their equipment and Sam Witherspoon for his advice and help in certain experiments. The advice and critical review of this manuscript by Joel Baseman is appreciated. The secretarial help of Jill Sapp and the efforts by Mrs. Kathy Mergele of the LaLeche League for obtaining for us the human breast milk are especially appreciated.

Received for publication 27 April 1984.

References

1. Ackers, J. P. 1982. Immunology of amebas, glardia, and trichomonads. *In* Immunology of Human Infection, Vol. 9, Part II, Viruses and Parasites; Immunodiagnosis and Prevention of Infectious Diseases. A. J. Nahmias and R. J. O'Reilly, editors. Plenum Publishing Corp., New York. 403–443.
2. Honigberg, B. M. 1978. Trichomonads of importance in human medicine. *In* Parasitic Protozoa II. J. P. Kreier, editor. Academic Press, New York. 275–392.
3. Spence, M. R., D. H. Hollander, J. Smith, L. McCaig, D. Sewell, and M. Brockman. 1980. The clinical and laboratory diagnosis of *Trichomonas vaginalis* infection. *Sex. Transm. Dis.* 7:168.
4. Kreiger, J. N. 1981. Urologic aspects of trichomoniasis. *Invest. Urol.* 18:411.
5. Alderete, J. F., and E. Pearlman. 1983. Pathogenic *Trichomonas vaginalis* cytotoxicity to cell culture monolayers. *Br. J. Vener. Dis.* 60:99.
6. Kreiger, J. N., M. A. Poisson, and M. F. Rein. 1983. Beta-hemolytic activity of *Trichomonas vaginalis* correlates with virulence. *Infect. Immun.* 41:1291.
7. Capron, A., J. P. Dessaint, D. Camus, and M. Capron. 1976. Some immune mechanisms in host-parasite relationships. *In* Biochemistry of Parasites and Host-Parasite Relationships. H. Van den Bossche, editor. Elsevier-North Holland Biomedical Press, Amsterdam. 263–282.
8. Bloom, B. R. 1979. Games parasites play: how parasites evade immune surveillance. *Nature (Lond.).* 279:21.
9. Whitnack, E., and E. H. Beachey. 1982. Antiopsonic activity of fibrinogen bound to M protein on the surface of group A streptococci. *J. Clin. Invest.* 69:1042.
10. Simonson, C., D. Brenner, and I. W. DeVoe. 1982. Expression of a high-affinity mechanism for acquisition of transferrin iron by *Neisseria meningitidis. Infect. Immun.* 36:107.

11. Bullen, J. J., H. J. Rogers, and E. Grifiths. 1978. Role of iron in bacterial infection. *Curr. Top. Microbiol. Immunol.* 80:1.
12. Peterson, K. M., J. B. Baseman, and J. F. Alderete. 1983. *Treponema pallidum* receptor binding proteins interact with fibronectin. *J. Exp. Med.* 157:1958.
13. Ouaissi, M. A., D. Afchain, A. Capron, and J. A. Grimaud. 1984. Fibronectin receptors on *Trypanosoma cruci* trypomastigotes and their biological function. *Nature (Lond.).* 308:380.
14. Brenner, D., I. W. DeVoe, and B. E. Holbein. 1981. Increased virulence of *Neisseria meningitidis* following in vitro iron-limited growth at low pH. *Infect. Immun.* 33:59.
15. Peterson, K. M., and J. F. Alderete. 1982. Host plasma proteins on the surface of pathogenic *Trichomonas vaginalis. Infect. Immun.* 37:755.
16. Peterson, K. M., and J. F. Alderete. 1983. Acquisition of α1-antitrypsin by a pathogenic stain of *Trichomonas vaginalis. Infect. Immun.* 40:640.
17. Peterson, K. M., and J. F. Alderete. 1984. Selective acquisition of plasma proteins by *Trichomonas vaginalis* and human lipoproteins as a growth requirement for this species. *Mol. Biochem. Parasitol.* In press.
18. Aisen, P. 1980. The transferrins. *In* Iron in Biochemistry and Medicine, Vol. 2. A. Jacobs and M. Worwood, editors. Academic Press, Inc., New York. 87–129.
19. Aisen, P., and A. Leibman. 1972. Lactoferrin and transferrin: a comparative study. *Biochem. Biophys. Acta.* 257:314.
20. Masson, P. L., J. F. Heremans, and C. H. Dive. 1966. An iron-binding protein common to many external excretions. *Clin. Chim. Acta.* 14:735.
21. Masson, P. L., J. F. Heremans, J. J. Prignot, and G. Wauters. 1966. Immunohistochemical localization and bacteriostatic properties of an iron-binding protein from bronchial mucus. *Thorax.* 21:538.
22. Masson, P. L., J. F. Heremans, and E. Schonne. 1969. Lactoferrin, an iron binding protein in neutrophilic leukocytes. *J. Exp. Med.* 130:643.
23. Marczak, R., T. E. Gorrell, and M. Müller. 1983. Hydrogenosomal ferredoxin of the anaerobic protozoan, *Tritrichomonas foetus. J. Biol. Chem.* 258:12427.
24. Müller, M., and T. E. Gorrell. 1983. Metabolism and metronidazole uptake in *Trichomonas vaginalis* isolates with different susceptibilities. *Antimicrob. Agents Chemother.* 24:667.
25. Budilova, M., and J. Kulda. 1977. Further studies on the effect of ferric ammonium citrate on the virulence of *Tritrichomonas foetus* to mice. *J. Protozool.* 24:49.
26. Diamond, L. S. 1968. Techniques of axenic culture of *Entomoeba histolytica schaudinni,* 1403 and *E. histolytica*-like amaebae. *J. Parasitol.* 54:1047.
27. Boeseman-Finkelstein, M., and R. A. Finkelstein. 1982. Sequential purification of lactoferrin, lysozyme and secretory immunoglobulin A from human milk. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 144:1.
28. Mickelsen, P. A., E. Blackman, and P. F. Sparling. 1982. Ability of *Neisseria gonorrhoeae, Neisseria meningitidis,* and commensal *Neisseria* species to obtain iron from lactoferrin. *Infect. Immun.* 35:915.
29. Garvey, J. S., N. E. Cremer, and D. H. Sussdorf. 1977. ^{125}I or ^{131}I-labeled proteins. *In* Methods in Immunology. D. H. Campbell, editor. W. A. Benjamin, Inc., Reading, MA. 171–182.
30. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature (Lond.).* 227:680.
31. Alderete, J. F. 1983. Identification of immunogenic and antibody-binding membrane proteins of pathogenic *Trichomonas vaginalis. Infect. Immun.* 40:284.
32. Cuatrecasas, P., M. Wilchek, and C. B. Antisen. 1968. Selective enzyme purification by affinity chromatography. *Proc. Natl. Acad. Sci. USA.* 61:636.

33. Feldman, H. A. 1972. Mathematical theory of complex ligand-binding systems at equilibrium: some methods for parameter fitting. *Anal. Biochem.* 48:317.
34. Proia, R. L., D. A. Hart, R. K. Holmes, K. V. Holmes, and L. Eidels. 1979. Immunoprecipitation and partial characterization of diphtheria toxin-binding glycoproteins from surface of guinea pig cells. *Proc. Natl. Acad. Sci. USA.* 76:685.