The Regulation by Iron of the Synthesis of Adhesins and Cytoadherence Levels in the Protozoan *Trichomonas vaginalis*

By Michael W. Lehker, Rossana Arroyo, and John F. Alderete

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

Summary

Levels of adherence of *Trichomonas vaginalis* to epithelial cells was found to be modulated by iron. Cytoadherence values were greater than or equal to twofold higher for trichomonads grown in a complex cultivation medium supplemented with iron. This increase in adherence levels was specifically mediated by iron; parasites cultured in a low-iron medium in the presence of salts other than iron were unresponsive to changes in adherence levels. Expression of the higher adherence property, by parasites grown first in low-iron medium followed by supplementation with iron, was a function of time, and the extent of cytoadherence was proportional to the concentration of iron added to the medium. Lactoferrin, an important iron source for trichomonads at the site of infection, elevated adherence of the parasite to epithelial cells, demonstrating the likely in vivo modulation of adherence by iron. The alteration of levels of adherence caused by iron was determined to be a reflection of gene expression of previously characterized trichomonad adhesins. Parasites grown under iron-replete conditions had higher quantities of surface-exposed adhesins, and this was a result of increased synthesis of adhesins. Actinomycin D and α-amanitin prevented expression of adhesin molecules, which resulted in decreased cytoadherence, showing that adhesin synthesis was dependent on gene transcription. Data indicated that genes encoding the four trichomonad adhesins are coordinately regulated by iron.

Specific adherence of mucosal pathogens to host cells leads to colonization, and this interaction is fundamental and prerequisite for infection and pathogenesis. Cytoadherence is an important mechanism by which infecting organisms overcome the flushing effect of mucosal secretions (1). *Trichomonas vaginalis* is a flagellated protozoan responsible for one of the most common, world-wide sexually transmitted diseases, and the parasite affects mostly women. The emotional and economic consequences to all world societies caused by this protozoan are significant.

This microorganism colonizes the vaginal epithelium by specific, receptor-ligand interactions (2). Adherence to squamous vaginal epithelial cells by this parasite is mediated by four surface proteins (3). The ability of *T. vaginalis* parasites to cytoadhere is the result of a complex cascade of events involving adhesin proteins (3) and proteinase activity (4).

These earlier studies were conducted with organisms grown in a complex, nutrient-rich medium different from that encountered by parasites in the vagina, a nutritional environment which itself is changing during the menstrual cycle. Recent continuous flow culture experiments demonstrated that *T. vaginalis* organisms were capable of altering several properties in response to varying culture environments (5). In addition, growth and multiplication (6), and certain virulence traits of *T. vaginalis*, were found to be modulated by iron, which is an essential nutrient for this parasite (6, 7). This ability of pathogenic human trichomonads to modify their physiology in response to different environments, especially those likely to be encountered in vivo, as may be the case with iron, prompted us to test whether iron might in fact regulate the important virulence property of cytoadherence.

This report shows that *T. vaginalis* had different levels of recognition and binding to epithelial cells when grown in a medium with varying concentrations of iron. Iron specifically regulated the synthesis of four trichomonad adhesin proteins, which when expressed on the surface of the parasite in greater amounts, resulted in elevated cytoadherence levels. Overall, our results indicate that genes encoding the adhesin proteins are coordinately regulated by iron. This may be the first report showing that iron controls the expression among pathogenic protozoa of important virulence genes, such as those encoding the adhesin proteins of *T. vaginalis*.

Materials and Methods

Parasite Growth and Radiolabeling of Proteins. *T. vaginalis* isolates used in this study were described previously (8, 9) and were culti-

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/91/08/0311/08 $2.00
Volume 174 August 1991 311-318

vated in a normal growth medium of trypticase-yeast extract-maltose (TYM)[1] supplemented with 10% heat-inactivated horse serum (10). Low-iron medium was prepared by the addition of 2,2-dipyridal (Sigma Chemical Co., St. Louis, MO) (0.15 mM final concentration) to growth medium. High-iron medium was made by the addition to growth medium of ferrous ammonium sulfate-hexahydrate (Sigma Chemical Co.) (0.25 mM final concentration) from a 100-fold stock solution made in 50 mM sulfosalicylic acid (6).

Radioiodination of membrane proteins was accomplished by the chloramine-T labeling technique, using 0.5 mCi of $Na^{125}I$ (sp. act., 3.7 GBq/ml) (Amersham Corp., Arlington Heights, IL) for radioiodination of 2×10^7 parasites, as described before (11), except that 1 mM *N*-α-*p*-tosyl-L-lysine chloromethyl ketone (TLCK) (Sigma Chemical Co.) was included in the labeling mixture and during washings. This was done to inhibit cysteine proteinases released by live trichomonads (12), which are known to degrade surface immunogens (13). Intrinsic radiolabeling with [^{35}S]methionine (sp. act., >37 GBq/mM) (ICN, Irvine, CA) was performed using standard conditions (8). For cytoadherence assays, trichomonads were also radiolabeled overnight (o/n) with 2 μCi/ml of [^{3}H]thymidine (sp. act., 64 GBq/mM) (ICN) (8). In this case, >80% of the radioactivity was precipitable with 5% trichloroacetic acid, showing the efficient incorporation of radioisotope into trichomonad macromolecules.

Host Cells. HeLa epithelial cells (American Type Culture Collection, Rockville, MD) were maintained in DMEM (Gibco-BRL, Gaithersburg, MD) with 10% FCS as described before (4, 14). Human vaginal epithelial cells were purified from vaginal swabs of healthy, uninfected women using a procedure recently described (2). Before use in the ligand assay described below, epithelial cells were fixed with 2.5% glutaraldehyde in PBS (10) for 1 h at 4°C as before (2, 3).

Cytoadherence and Ligand Assay. The measurement of the extent of *T. vaginalis* cytoadherence to HeLa cells was by a modification of a previously standardized procedure. HeLa cells (10^5) were seeded into each well of a 96-well microdilution plate and incubated for 4 h at 37°C. Nonadherent cells were removed by washing the monolayer three times with DMEM warmed to 37°C. A 100-μl sample containing 5×10^5 [^{3}H]thymidine-labeled parasites suspended in a 2:1 (vol/vol) DMEM/TYM mixture without serum found suitable for both trichomonads and host cells (14) was then added to the HeLa cell monolayers. After incubation for up to 30 min at 37°C in a 7% CO_2 atmosphere, unbound parasites were removed by washing with warm DMEM. After the plates dried, the level of cytoadherence was determined by counting individual wells by scintillation spectroscopy. Measurement of trichomonal cytoadherence to vaginal epithelial cells was also by a recently detailed procedure (2).

The ligand assay for identification of adhesins was by a modification of an earlier published method (3). Parasites (2×10^7) were solubilized in 0.5 ml of PBS-1% deoxycholate (Sigma Chemical Co.) containing 1 mM TLCK. To this extract was added 0.5 ml of TDSET (10 mM Tris-HCl, pH 7.0, 10 mM EDTA, 0.2% deoxycholate, 0.1% SDS, and 1% Triton X-100) buffer also containing 1 mM TLCK. After centrifugation at 100,000 *g*, the supernatant (~1 ml) was added to fixed HeLa cells (10^6) or vaginal epithelial cells (10^6) and incubated for 18 h at 4°C. Fixed host cells were then centrifuged at 600 *g* and washed three times with TDSET Buffer. Finally, fixed host cells were boiled in electrophoresis dissolving buffer (15) for 3 min and released parasite proteins subjected to SDS-PAGE using 7.5% or 10% separating acrylamide gels.

Preparation of Iron-binding Proteins. Lactoferrin and transferrin (both from Sigma Chemical Co.) (1 mg/ml) were dissolved in 100 mM sodium citrate-100 mM carbonate buffer, pH 8.6, and saturated with iron by addition of 50-fold molar excess of $FeCl_3$. Free iron was removed by chromatography on a 1 × 15-cm Sephadex G-25 (Pharmacia Fine Chemicals, Piscataway, NJ) column. Apotransferrin and apolactoferrin were prepared by incubating the proteins o/n at 4°C in a solution of 100 mM sodium acetate-100 mM sodium phosphate-25 mM EDTA, pH 5.2, before chromatography through Sephadex G-25 (16). The degree of iron saturation of these proteins was determined spectrophotometrically at an absorbance of 465 nM (17).

Antibodies. Each of four rabbits were individually immunized with mascerated acrylamide gel slices containing one of the four adhesins from a preparative ligand assay (3). The acrylamide-adhesin protein mixture was then injected intramuscularly. Booster immunizations were administered 2 wk later and at monthly intervals. Specific antibody was evidenced by immunoblot detection of adhesins from the ligand assay and compared with reactions using blots of total parasite proteins (13). The IgG fraction of each antiadhesin serum was purified by protein A–Sepharose affinity chromatography (Pharmacia Fine Chemicals). Each antiadhesin IgG reacted with only the respective adhesin and, as expected, inhibited cytoadherence (3). Purified IgG from prebled sera of all rabbits was used as negative controls in duplicate immunoblot or cytoadherence inhibition experiments handled identically.

SDS-PAGE and Immunoblot. SDS-PAGE of trichomonad proteins and electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose (NC) for immunoblotting have been described (18, 19). Molecular weight markers were from BioRad Laboratories (Richmond, CA).

NC blots were blocked with 5% nonfat dry milk (NFDM) in TBS (20 mM Tris-HCl, pH 7.4, and 500 mM NaCl) for 2 h at room temperature (RT). A 1:10 dilution of rabbit antiadhesin serum prepared in NFDM-TBS was added, and blots were then incubated o/n at RT. Goat anti-rabbit Ig conjugated to horseradish peroxidase (BioRad Laboratories) was finally added (dilution of 1:1,500 in NFDM-TBS-0.05% Tween 20). The blots were incubated o/n at 4°C. After washing blots three times for 10 min with TBS-0.05% Tween 20, blots were treated with 4-chloro-1-naphtol (2 mg/ml) prepared in TBS-20% methanol containing 0.015% H_2O_2.

Results

Levels of Adherence to Host Cells by Long-term Grown and Fresh T. vaginalis Isolates Are Modulated by Iron. A representative long-term-grown laboratory isolate, NYH 286, and a fresh isolate, T048, of *T. vaginalis* were examined for kinetics of cytoadherence to epithelial cells. These isolates were cultivated in the complex normal growth medium modified for high and low concentrations of iron. The kinetics of cytoadherence were similar for both isolates (Fig. 1 *A*); maximal adherence was achieved within 15 min. Higher levels of cytoadherence were reproducibly seen by trichomonads grown in high-iron medium, when compared with parasites deprived of iron. For isolate NYH 286, the parasite-to-cell (P/C) ratio, which is a value based on the number of trichomonads adherent to the cell monolayer and therefore useful for com-

[1] *Abbreviations used in this paper:* NC, nitrocellulose; NFDM, nonfat dry milk; o/n, overnight; P/C, parasite-to-cell; RT, room temperature; TLCK, *N*-α-*p*-tosyl-L-lysine chloromethyl ketone; TYM, trypticase-yeast extract-maltose.

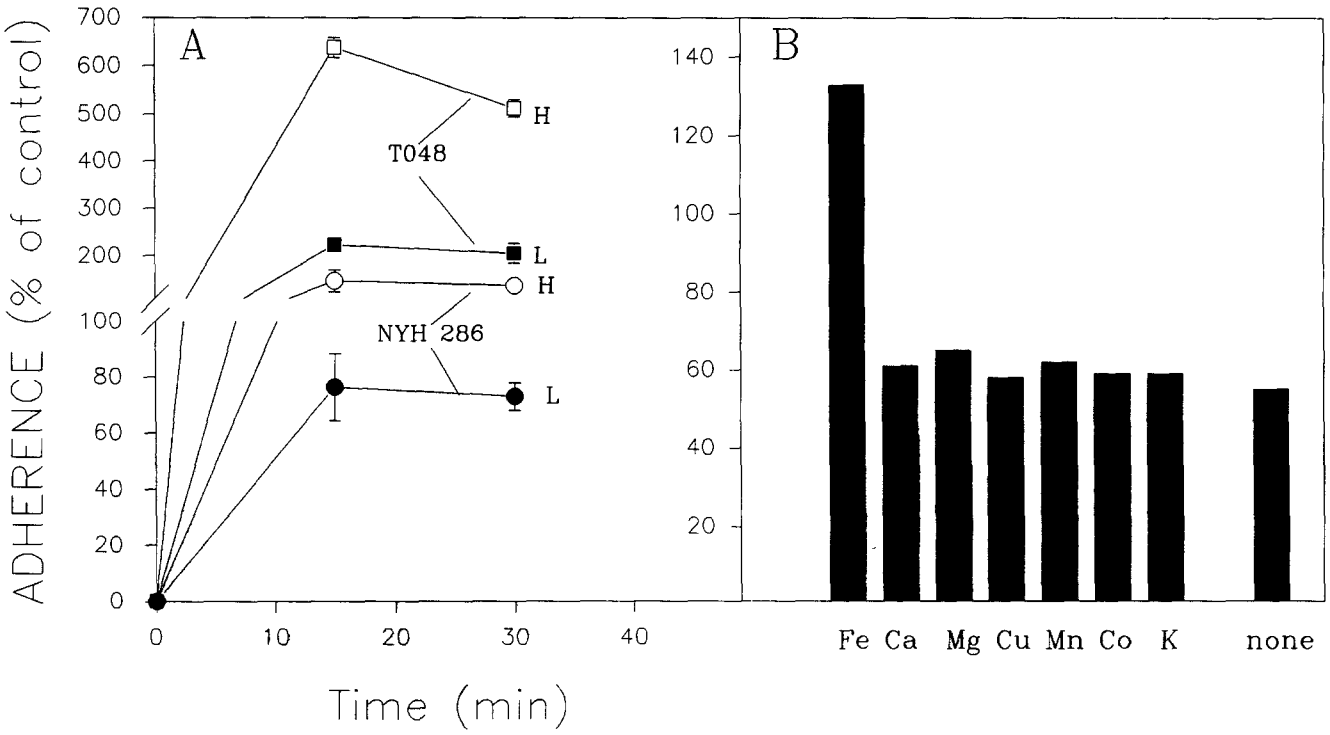

Figure 1. Cytoadherence kinetics (*A*) of *T. vaginalis* in high- (H) and low-iron medium (L) and specificity of iron-modulated cytoadherence (*B*). Cytoadherence assays were performed in 96-well microtiter plates. Parasites (5×10^5) radiolabeled with [^{3}H]thymidine in 100 μl of a medium mixture of TYM-DMEM (2:1; vol/vol) were added to confluent HeLa cell monolayers. After interaction, nonadherent trichomonads were removed by washing, and associated radioactivity of the wells was determined by scintillation spectroscopy. For cytoadherence kinetics (*A*) *T. vaginalis* isolate NYH 286 and T048 were grown to late-logarithmic growth phase in high- (●, ■) or low-iron (○, □) medium. The specificity of iron-modulated cytoadherence (*B*) was determined by measuring the level of cytoadherence of low-iron grown trichomonads (NYH 286) after additional growth in TYM serum medium supplemented with 250 μM cationic salts. Abbreviations: Fe, ferrous ammonium sulfate; Ca, calcium chloride; Mg, magnesium chloride; Mn, manganese chloride; Co, cobalt chloride; Cu, cupric sulfate; K, potassium chloride. Cytoadherence values are relative to the level of adherence of *T. vaginalis* NYH 286 in normal TYM serum medium, which was set as 100% for comparative purposes.

parative purposes, as has been described before (2), was 1.75 and 0.80 for high- and low-iron grown trichomonads, respectively, at the 15-min time points. These P/C values were consistent with previously published values (14), showing the reproducibility of the adherence conditions and assay. For this assay, neither the presence nor absence of iron or the iron chelator in the DMEM-TYM medium mixture for the adherence assay affected the level of cytoadherence.

P/C ratios for the fresh clinical isolate, T048, were 8.75 and 3.0, illustrating the much higher cytoadherence capability of the fresh isolate, T048, compared with NYH 286, the common laboratory isolate (Fig. 1 *A*). A comparison of numerous other fresh and long-term grown isolates gave similar data (Table 1); all fresh isolates yielded higher overall levels of cytoadherence. It is noteworthy that all isolates, regardless of whether grown for short or long periods of time, were capable of responding to the iron in the medium. The range of percent differences in cytoadherence between organisms grown in high- vs. low-iron medium ranged from 14% to 67%. A few long-term grown isolates, though, were relatively unresponsive, as seen for isolates IR 78 and JH 31A. These results show that isolates that have been passaged in vitro for several years are no longer representative of wild type, infecting *T. vaginalis* parasites in their overall level of cytoadherence and responsiveness to iron.

The specificity of iron modulation of cytoadherence was then tested, and results from a typical experiment are presented in Fig. 1 *B*. Addition of other cationic salts to trichomonads grown in the low-iron medium failed to restore levels of cytoadherence to those seen for parasites under the same conditions but after addition of iron. Trichomonads were highly motile and fully viable in these experiments, showing that these cationic salts were not toxic to the parasites and reaffirming the specificity of iron in regulating cytoadherence.

Relationship between Iron Concentration in Growth Medium and Extent of Cytoadherence. We wanted to continue to examine the specificity in iron modulation of the levels of cytoadherence by determining the concentration of iron needed for optimal expression of host parasitism by trichomonads. To do this we needed to know the time required for induction of adherence in low-iron-grown parasites transferred to high-iron medium. Parasites first grown in low-iron medium, washed, and suspended in high-iron medium required 30 min before any increase in cytoadherence levels was detectable (Fig. 2). The increase continued for an additional 60 min. No change in the already low level of cytoadherence was seen for para-

Table 1. *Examination of T. vaginalis Isolates Grown in High- and Low-iron Medium for Levels of Cytoadherence*

Isolate‡	Relative adherence value*		
	High iron	Low iron	Percent reduction
Fresh:			
T038	4.96 ± 0.39	2.08 ± 0.23	58.0
T048	6.53 ± 0.84	2.16 ± 0.32	66.9
T023	2.31 ± 0.21	1.58 ± 0.17	31.6
T036	2.75 ± 0.31	1.82 ± 0.16	33.8
Long-term grown:			
NYH 286	1.19 ± 0.14	0.58 ± 0.04	51.0
T005	1.46 ± 0.21	0.72 ± 0.12	50.6
T003	1.52 ± 0.14	0.96 ± 0.06	37.0
RU 375	1.50 ± 0.11	1.10 ± 0.09	26.0
IR 78	0.73 ± 0.08	0.60 ± 0.12	14.2
JH 31A	0.95 ± 0.12	0.78 ± 0.15	17.8

* Adherence of NYH 286 grown in normal complex growth medium was set as 1 for comparative purposes, as has been done before (4), to show the greater levels of cytoadherence by fresh isolates.
‡ Isolates grown for >30 d in vitro were considered long-term grown. Fresh clinical isolates were grown for <30 d in vitro (9). *T. vaginalis* organisms were grown in high- or low-iron medium (see Materials and Methods) in the presence of 2 μCi/ml [^{3}H]thymidine. At the late logarithmic growth phase, which occurred between 24 and 48 h of growth, parasites were washed twice and added to confluent HeLa cell monolayers in microtiter wells, as described in Materials and Methods. Nonadherent trichomonads were removed by washing and associated radioactivity of the wells counted by scintillation spectroscopy.

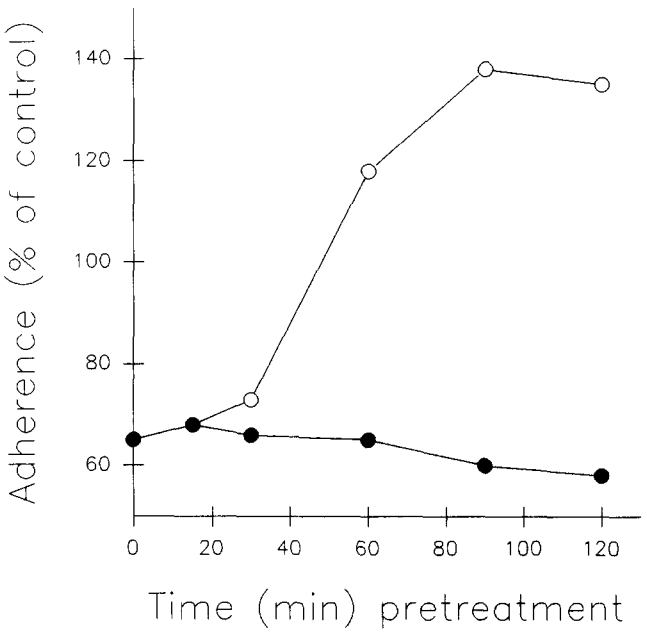

Figure 2. Time-dependent restoration of cytoadherence by iron after iron-limited growth. *T. vaginalis* isolate NYH 286 was grown in iron-limited medium and then resuspended to a density of 10^6 parasites/ml in medium containing 250 μl ferrous ammonium sulfate (○), or 100 μl 2,2-dipyridal (●). At 15-min intervals, parasites were removed, washed, and the percent attachment to HeLa cell monolayers in microtiter wells was determined. Cytoadherence of *T. vaginalis* NYH 286 grown in normal TYM serum medium was set as 100% for comparative purposes.

sites of low-iron medium transferred again to a low-iron environment.

This relative short induction period now allowed us to test the effect of various iron concentrations on cytoadherence levels by using a 2-h time period. Trichomonads were again removed from the low-iron medium, washed, and transferred into test tubes containing growth medium with varying concentrations of iron. After 2 h, a time in which medium pH and other physiologic parameters remained unaltered, the level of cytoadherence was found to be solely dependent on the concentration of iron in the medium (Fig. 3). Maximal cytoadherence was obtained at 100 μM iron, and the levels reached at the other iron concentrations also reflected maximum cytoadherence capabilities of the parasite, since longer incubation times beyond 2 h did not increase further the extent of adherence.

Elevated Cytoadherence Is Due to Increased Synthesis and Surface Expression of Trichomonad Adhesins. Four trichomonad proteins with relative molecular masses of 65 kD (AP65), 51 kD (AP51), 33 kD (AP33), and 23 kD (AP23) have been identified as the trichomonad adhesins (3). It became necessary, therefore, to attempt to understand the molecular basis for the iron modulation of cytoadherence by analyzing the amounts of adhesins synthesized under the different growth conditions.

Fig. 4 *A* shows the autoradiographic profiles of the adhesins obtained from a ligand assay in which a detergent extract of surface-labeled parasites was incubated with fixed HeLa cells (3) (see Materials and Methods). The four iodinated adhesins were present in larger amounts when the ligand assay was performed with organisms grown in a high-iron medium (lane *H*) when compared with parasites grown in either a normal growth medium (lane *N*) or a low-iron medium (lane *L*). The four adhesins were present in the normal- and low-iron medium, albeit in decreased amounts, and were barely detectable in autoradiograms of experiments performed simultaneously and under identical conditions. Visualization of iodinated adhesins in the normal- and low-iron-grown trichomonads required a longer exposure of the gel to X-ray film for this ligand assay.

These data were further reinforced by immunoblot experiments in which the adhesins were from a ligand assay performed simultaneously as above. After isolation of the adhesins from the fixed cells and electrophoresis and blotting, adhesins on NC were probed with a pooled preparation of each monospecific antiadhesin serum. The four adhesins were readily detected in parasites grown in high-iron medium (Fig. 4 *A2*, lane *H*), but not in blots with adhesins from organisms grown in normal (lane *N*) or low-iron medium (lane *L*).

It was important then to see whether iron influenced overall

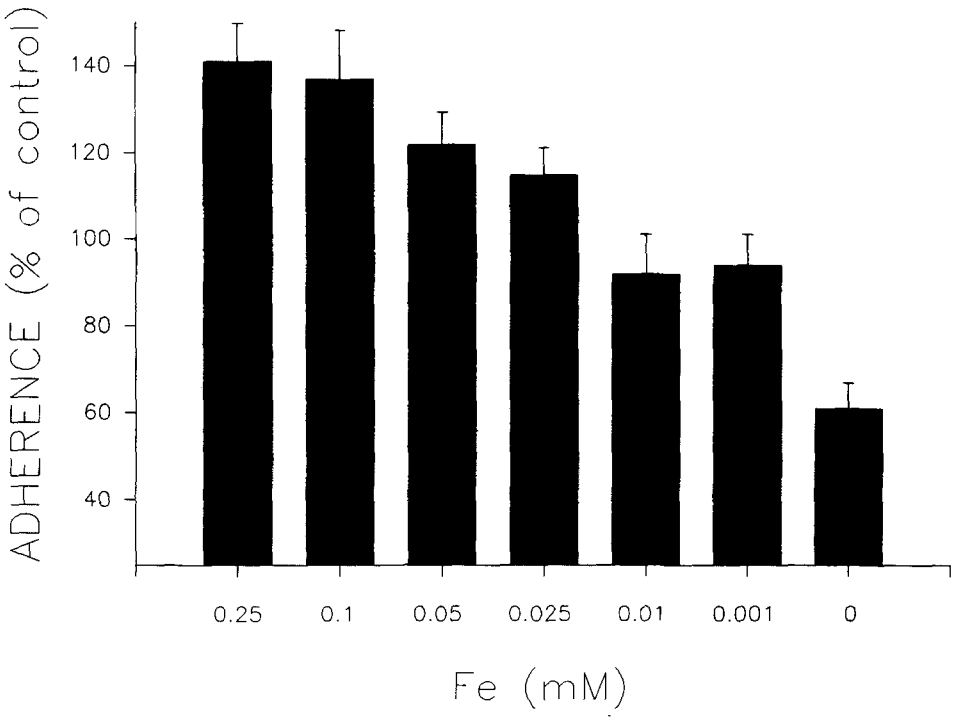

Figure 3. *T. vaginalis* NYH 286 cytoadherence dependence on iron concentration. Trichomonads at the late logarithmic phase of growth in low-iron medium were washed and resuspended in low-iron medium supplemented with various concentrations of iron. After a 2-h incubation period, the extent of parasite cytoadherence was determined as described in Materials and Methods.

adhesin synthesis or only surface expression of adhesins. Total synthesis of the adhesins was monitored in a ligand assay using detergent extracts of [^{35}S]methionine-labeled trichomonads. Fig. 4 *B* illustrates the difference in total adhesin amounts obtained from parasites cultivated in the normal growth medium with iron added at varying concentrations. Synthesis of adhesins was dependent on the concentration of iron, and again organisms from a high-iron medium (lane *H*) had the most intense bands on fluorograms, indicative of the highest amounts of adhesins. These data are in accord with the cytoadherence data shown in Fig. 3. It was especially noteworthy that parasites grown in the high- and low-iron medium had similar overall protein synthesis patterns (Fig. 4 *C*), since fluorograms of total proteins were equally complex and comparable for both high-iron and low-iron-medium-grown organisms. These data demonstrate the specificity of the regulation by iron of only the adhesins, and not all proteins. As expected, supplementation of low-iron medium with divalent cations other than iron did not give any enhancement of the amounts of adhesins over those seen above for low-iron grown parasites (Fig. 4, lane *L*) (data not shown).

Expression of Adhesins for Increased Iron-mediated Cytoadherence Is Dependent upon Transcription and Translation. Levels of cytoadherence and amounts of adhesins were analyzed for low-iron-grown parasites transferred to high-iron medium containing cycloheximide (protein synthesis inhibitor), and

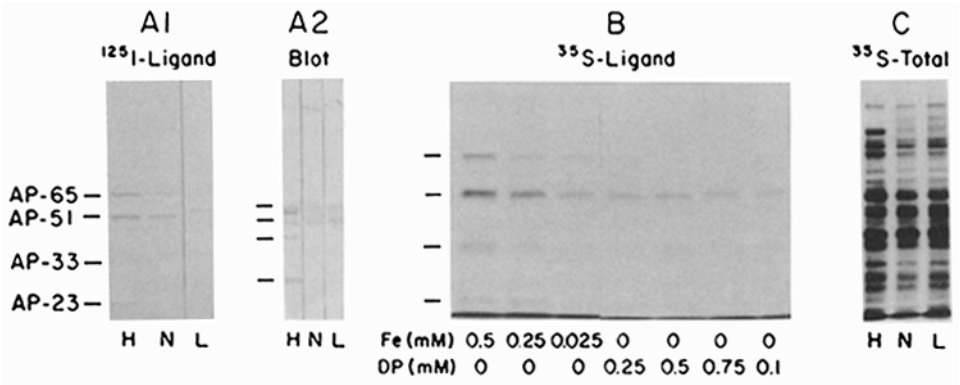

Figure 4. Iron modulation of surface expression and synthesis of the four trichomonad adhesins (3). *T. vaginalis* T048 was grown in high- (H) or low- (L) iron medium or in normal growth medium (N). Parasites were then used for a ligand assay to identify the adhesins as described in Materials and Methods, and acrylamide gels were analyzed by autoradiography using ^{125}I surface-labeled parasites (*A1*) and by fluorography using [^{35}S]methionine-labeled trichomonads (*B*) grown in TYM serum media of different iron-content. Duplicate gels of the experiment performed in *A1* were also blotted and probed using a pooled preparation of monospecific antiadhesin serum (*A2*). The fluorogram of total proteins of parasites grown in high-, normal-, and low-iron medium is shown in *C*.

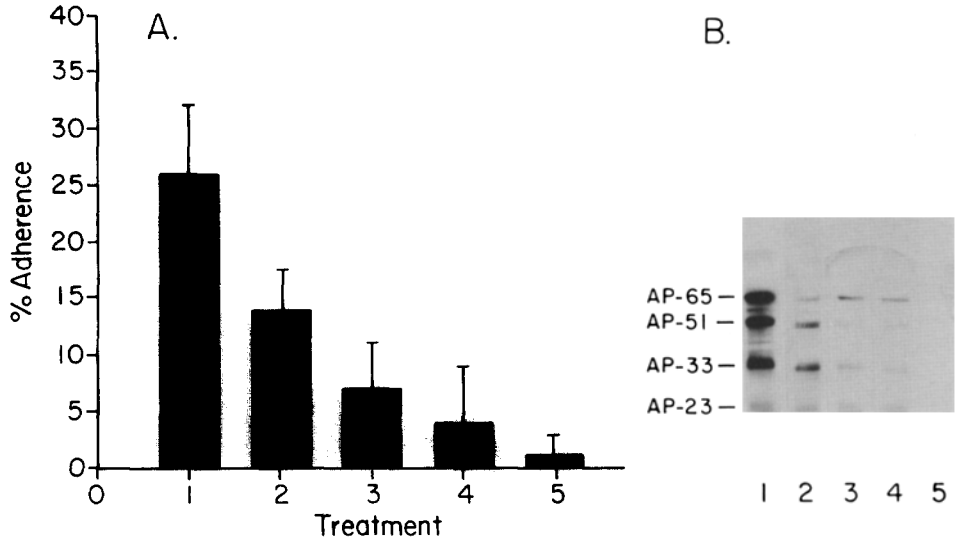

Figure 5. Inhibitors of protein synthesis and transcription abrogate trichomonal cytoadherence and expression of adhesins. *T. vaginalis* T048 was grown in low-iron medium, and parasites were washed and resuspended to a density of 10^6 and incubated for 8 h in high-iron medium without additions (lane *1*), with 10 μg/ml actinomycin D (lane *3*), with 10 μg/ml α-amanitin (lane *4*) with 10 μg cycloheximide (lane *5*), or in low-iron medium (lane *2*). Cytoadherence of parasites to HeLa cells after treatment was measured as described in Materials and Methods.

α-amanitin or actinomycin D (transcription inhibitors). As seen in Fig. 5 *A*, these inhibitors greatly reduced and, in the case of cycloheximide, abolished the ability of trichomonads to cytoadhere, especially when compared with cytoadherence readily detectable in the absence of inhibitors in the medium. Duplicate samples of [^{35}S]methionine-labeled parasites handled identically were used for a ligand assay, and fluorograms show that these inhibitors also shut down synthesis of adhesins (Fig. 5 *B*). These data provide strong evidence that iron regulates the gene expression of adhesins. Surprisingly, inhibitors caused the parasites to have levels of cytoadherence and amounts of adhesins lower than even trichomonads grown in low-iron medium, indicating that continuous synthesis of adhesins is required for maintenance of specific cytoadherence.

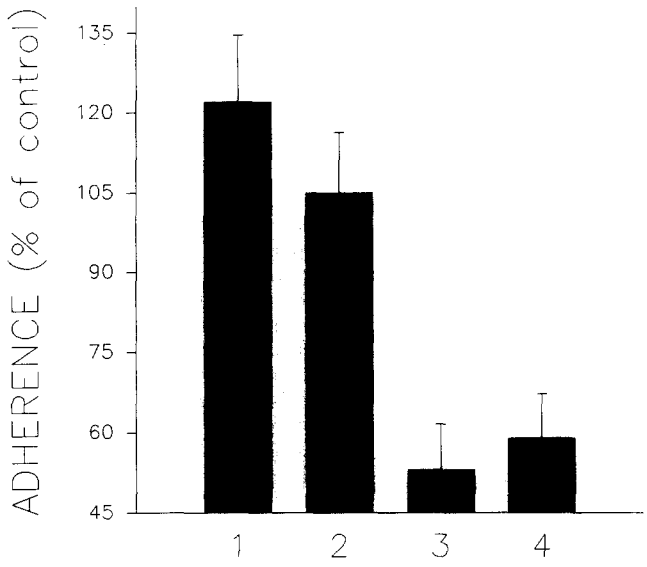

Figure 6. Lactoferrin as a source of iron to elevate cytoadherence levels. Trichomonads were grown in TYM serum medium containing either 250 μM iron (lane *1*), iron saturated human lactoferrin (1 mg/ml) (lane *2*), apolactoferrin (1 mg/ml) (lane *3*), or 2,2 dipyridal (0.1 mM) (lane *4*). Adherence to vaginal epithelial cells was determined as described before (2), by microscopic enumeration.

Lactoferrin Regulates Adherence and Adhesins. Our final experiment was an attempt to show that iron modulation of cytoadherence and adhesins may also occur in vivo. For this set of experiments, lactoferrin, the host iron-binding protein of vaginal secretions, known to be a source of iron for this parasite (16) and vaginal epithelial cells, the target host cell, were used. Fig. 6 shows the increased attachment to the vaginal epithelial cells by a fresh isolate grown in low-iron medium supplemented with iron-saturated lactoferrin (sample 2), like that seen for iron-supplemented medium (sample 1). In contrast, transferrin, an iron-binding protein, which is not a source of iron for this organism (16), gave no increase in cytoadherence, as did apolactoferrin (sample 3). These data demonstrate that this parasite can indeed modulate cytoadherence in response to the iron source found in vivo. In data not shown, the amounts of adhesins, as presented in Fig. 4, were altered accordingly.

Discussion

It is evident that *T. vaginalis*, like many other microorganisms, undergoes differential gene expression depending on iron concentrations and availability, in addition to a variety of other physiological conditions (5, 7). Iron was recently shown to regulate the expression of genes encoding for the lactoferrin receptor and also the expression of genes for numerous other immunogens (7). This study represents an effort to extend our understanding of the function of iron-regulated proteins of *T. vaginalis*, especially those directly involved in host parasitism, and therefore, virulence.

Cytoadherence in *T. vaginalis* is multifactorial, as it requires proteinase activity (4) and receptor-ligand interactions (3). We now show that iron specifically modulates cytoadherence of trichomonads to target cells, making this parasite change its adherence properties depending on the growth environment, as occurs for other microbial systems (20–24). In this

case, cytoadherence levels were greater than two times higher for trichomonads grown in iron-supplemented medium than in low-iron medium (Fig. 1), and this was a direct result of synthesis of adhesins for placement on the surface of parasites. The relationship between iron concentrations, cytoadherence levels (Table 1), and increased adhesion expression (Fig. 4) in an iron-replete medium was found to be dependent on gene transcription and translation (Fig. 5). Overall, these data strongly indicate that iron coordinately regulates trichomonad adhesin gene expression.

Examples exist for iron to exert positive or negative control on gene transcription in bacteria (25, 26) or mammalian cells (27, 28). The data presented in this report indicate that trichomonad adhesin genes are positively regulated by iron, as demonstrated by the increased synthesis of adhesins in the presence of iron (Fig. 4) and the requirement for gene transcription (Fig. 5). Although the mechanism of control is unknown, it is likely to consist of a set of reactions involving iron, protein, and DNA interactions similar to other iron-regulated systems (25).

It is noteworthy that long-term isolates have significantly lower cytoadherence levels than fresh clinical isolates (Table 1), suggesting that extended in vitro cultivation in the complex medium alters trichomonal regulation of important virulence genes, like those of the adhesins. Laboratory cultivation may result in inappropriate environmental signaling. Alternatively, parasites may be selected that do not resemble those during clinical disease, and they may be incapable of responding to the same regulatory machinery for gene expression as fresh isolates. Undoubtedly, these variables have contributed to discrepancies in the literature about the virulence potential among isolates. It is now clear that long-term grown trichomonads are of limited value to achieve a precise molecular understanding of trichomonal virulence. It may be fortuitous that in vitro cultivation yields some isolates that are unresponsive to the same regulatory elements as wild-type parasites, thereby allowing for the future dissection and understanding of gene regulatory mechanisms for *T. vaginalis*.

An important question is whether the observations presented here on iron-regulated adhesin expression occur in vivo. In the absence of a suitable animal model and with the difficulty in analyzing patient material, the question cannot be answered directly. Trichomonads grown in medium supplemented with lactoferrin (Fig. 6), a major iron source for this parasite (16) and the major iron-binding protein in vaginal secretions (29), have elevated adhesins. It has also been demonstrated that trichomonads from patients indeed are representative of both low- and high-iron-grown organisms (7). The analysis for the expression of adhesins of trichomonal populations directly from fresh isolates is now of special interest, since the possibility that iron-regulated adhesin expression occurs in vivo seems likely based on the ability of this organism to sequester iron through specific, receptor-mediated binding of lactoferrin (16) and other iron-containing proteins (30).

Regulating adhesins may be a necessity for survival by this protozoan. This property may allow the parasite to adapt to changes in the vagina during the menstrual cycle. In response to hormonal levels, several types of epithelial cells prevail at different times of the menstrual phase, and desquamation of vaginal epithelial cells occurs (31). Similarly, lactoferrin concentrations in vaginal fluids are known to undergo dramatic changes during menstrual cycle. Amounts of lactoferrin are elevated after the postmenstrual phase and steadily decline until menstruation (29). Thus, iron availability from lactoferrin may be the environmental signal that triggers trichomonads to modulate amounts of adhesins, thereby permitting the recognition and binding to the prevailing host epithelial cell. Even more importantly, given the high requirement for iron by this organism (6), low-iron conditions at the site of infection and the downregulation of adhesin synthesis may result in the ability of trichomonads to migrate toward more iron-rich sites. The end result of such a strategy would be the persistence of this protozoan within the vagina, despite the flushing action of mucosal secretions, desquamation of the mucosal epithelium, and an overall nutrient limitation. Once again, the tremendously complex nature of this host-parasite interrelationship is illustrated.

The excellent secretarial assistance of Diana Hinojosa is especially acknowledged.

This study was supported by U.S. Public Health Service grant AI-18768 from the National Institute of Allergy and Infectious Disease, National Institutes of Health. M. W. Lehker was supported in part by NIH Training grant T32-AI-07271.

Address correspondence to John F. Alderete, Department of Microbiology, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78284.

Received for publication 26 March 1991.

References

1. Abraham, S.N., and E.H. Beachey. 1985. Host defenses against adhesion of bacteria to mucosal surfaces. *In* Advances in Host Defense Mechanisms, Volume 4, Mucosal Immunity. J.I. Gallin and A.S. Fauci, editor. Raven Press, New York. 63–88.
2. Alderete, J.F., P. Demeš, A. Gombošova, M. Valent, M. Fabošova, A. Janoška, J. Štefanović, and R. Arroyo. 1988.

Specific parasitism of purified vaginal epithelial cells by *Trichomonas vaginalis*. *Infect. Immun.* 56:2558.
3. Alderete, J.F., and G.E. Garza. 1988. Identification and properties of *Trichomonas vaginalis* proteins involved in cytadherence. *Infect. Immun.* 56:28.
4. Arroyo, R., and J.F. Alderete. 1989. *Trichomonas vaginalis* proteinase activity is necessary for parasite cytadherence. *Infect. Immun.* 57:2991.
5. Lehker, M.W., and J.F. Alderete. 1990. Properties of *Trichomonas vaginalis* grown under chemostat growth conditions. *Genitourin. Med.* 66:193.
6. Gorrell, T.E. 1985. Effect of culture medium iron content on the biochemical composition and metabolism of *Trichomonas vaginalis*. *J. Bacteriol.* 161:1228.
7. Lehker, M.W., and J.F. Alderete. 1991. Iron regulates several *Trichomonas vaginalis* virulence properties and expression of protein immunogens. *J. Mol. Microbiol.* In press.
8. Alderete, J.F. 1983. Antigen analysis of several pathogenic strains of *Trichomonas vaginalis*. *Infect. Immun.* 39:1041.
9. Neale, K.A., and J.F. Alderete. 1990. Analysis of the proteinases of representative *Trichomonas vaginalis* isolates. *Infect. Immun.* 58:157.
10. Peterson, K.M., and J.F. Alderete. 1982. Host plasma proteins on the surface of pathogenic *Trichomonas vaginalis*. *Infect. Immun.* 37:755.
11. Alderete, J.F. 1983. Identification of immunogenic and antibody-binding membrane proteins of pathogenic *Trichomonas vaginalis*. *Infect. Immun.* 40:284.
12. Lockwood, B.C., M.J. North, and G.H. Coombs. 1987. The release of hydrolases from *Trichomonas vaginalis* and *Tritrichomonas foetus*. *Mol. Biochem. Parasitol.* 30:135.
13. Neale, K.A., and J.F. Alderete. 1989. Relatedness of structure of a major immunogen in *Trichomonas vaginalis* isolates. *Infect. Immun.* 57:1849.
14. Alderete, J.F., and G.E. Garza. 1985. Specific nature of *Trichomonas vaginalis* parasitism of host cell surfaces. *Infect. Immun.* 50:701.
15. Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature (Lond.).* 227:680.
16. Peterson, K.M., and J.F. Alderete. 1984. Iron uptake and increased intracellular enzyme activity follow lactoferrin binding by *Trichomonas vaginalis* receptors. *J. Exp. Med.* 160:398.
17. Mazurier, J., and G. Spik. 1980. Comparative study of the iron-binding properties of human transferrins. I. Complete and sequential iron saturation and desaturation of lactoferrin. *Biochim. Biophys. Acta.* 629:399.
18. Tobwin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins. *Proc. Natl. Acad. Sci. USA.* 76:4350.
19. Alderete, J.F., L. Suprun-Brown, L. Kasmala, J. Smith, and M. Spence. 1985. Heterogeneity of *Trichomonas vaginalis* and discrimination among isolates and subpopulations with sera of patients and experimentally infected mice. *Infect. Immun.* 49:463.
20. Lee, C.A., and S. Falkow. 1990. The ability of Salmonella to enter mammalian cells is affected by bacterial growth state. *Proc. Natl. Acad. Sci. USA.* 87:4304.
21. Kennedy, M.J., A.L. Rogers, and R.J. Yancey. 1989. Environmental alteration and phenotypic regulation of *Candida albicans* adhesion to plastic. *Infect. Immun.* 57:3876.
22. Williams, P.H., and G. Hinson. 1987. Temperature-dependent transcriptional regulation of expression of fimbriae in an *Escherichia coli* strain isolated from a child with severe enteritis. *Infect. Immun.* 55:1734.
23. Eisenstein, B.I., and D.C. Dodd. 1982. Pseudocatabolite repression of type 1 fimbriae of *Escherichia coli*. *J. Bacteriol.* 151:1560.
24. Avots-Avotins, A.E., R.C. Fader, and C.P. Davis. 1981. Environmental alteration and two distinct mechanisms of *E. coli* adherence to bladder epithelial cells. *Invest. Urol.* 18:364.
25. Hennecke, H. 1990. Regulation of bacterial gene expression by metal-protein complexes. *Mol. Microbiol.* 4:1621.
26. Miller, J.F., J.J. Mekalanos, and S. Falkow. 1989. Coordinate regulation and sensory transduction in the control of bacterial virulence. *Science (Wash. DC).* 243:916.
27. Theil, E.C. 1990. Regulation of ferritin and transferrin receptor mRNAs. *J. Biol. Chem.* 25:4771.
28. Worwood, M. 1989. An overview of iron metabolism at a molecular level. *J. Intern. Med.* 226:381.
29. Cohen, M.S., B.E. Britigan, M. French, and K. Bean. 1987. Preliminary observations on lactoferrin secretion in human vaginal mucus: Variation during the menstrual cycle, evidence of hormonal regulation and implications for infection with *Neisseria gonorrhoea*. *Am. J. Obstet. Gynecol.* 157:1122.
30. Lehker, M.W., T.H. Chang, D.C. Dailey, and J.F. Alderete. 1990. Specific erythrocyte binding is an additional nutrient acquisition system for *Trichomonas vaginalis*. *J. Exp. Med.* 171:2165.
31. Lopez, Colombo de Allende, I. 1950. Cytology of the human vagina. I. L.C. de Allende and O. Orias, editors. Hoeber, New York. 42–67.