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Estrogen Actions on Trichomonas vaginalis, Tritrichomonas foetus, and Trichomonas gallinae

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ABSTRACT

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The hormonal milieu can alter susceptibility to infection. The effect of hormones on Trichomonas vaginalis, Tritrichomonas foetus, and Trichomonas gallinae, was studied using axenically cultured clinical isolates. Estrogens in physiological concentrations, decreased the growth of T. vaginalis and adherence to McCoy cells in vitro, and acted as a chemorepellent. The specificity of these effects was verified by their being blocked with tamoxifen, an anti-estrogen, by the dose and time dependency of the responses, and by the lack of effect with other hormones. T. gallinae and T. foetus were not responsive to estrogens. Estrogen binding site determinations showed all isolates displayed specific estrogen binding with similar binding affinity (Ka, Kd), however there were significant differences in estrogen binding capacities comparing T. vaginalis with T. gallinae and T. foetus. T. vaginalis displayed significantly greater total estrogen binding capacity compred with the non-estrogen responsive trichomonads. These results show that estrogens directly decrease the virulence of T. vaginails, however, interactions between estrogens and mammalian cells have a different effect and promote the development of infection. Complicated interactions between hormones, microorganisms, and mammalian cells ultimately determine whether such exposure predisposes to or prevents the development of infection.

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Introduction

Trichomonas vaginalis, the causative agent of human genital trichomoniasis was first described by A. Donne' in 1836[1]. Yet after a century and a half, many questions regarding the pathogenicity of this organism remain unanswered. A typical human infection by the parasite lacks easily discernible features associated with many other infectious diseases, and symptomless chronic infections are known to occur. The more subtle characteristics of the disease have not been studied in an experimental host, because infection corresponding to that in humans has not been reproduced despite attempts with a number of animal species.

Trichomonas vaginalis has been cultivated with varied success under conditions too numerous to cite. None of the culture methods can sufficiently distinguish between virulent and avirulent strains. The classification of isolates as pathogenic or nonpathogenic is based soley on the severity of the symptoms of the patients from whom the particular strains were recovered. There is no other direct procedure by which the pathogenicity of the organisms can be determined.

Infection may elicit a profuse, acute, inflammatory discharge containing many polymorphonuclear neutrophils and trichomonads. Numerous imidazole compounds have activity against Trichomonas vaginalis, and occasional reports of resistance to these compounds (e.g. the 5-nitroimidazole metronidazole) explain some cases of clinical failure[2]. Because treatment failures seem to be far more

common than the presence of resistant organisms other factors related to the virulence of these organisms should be considered.

Observations of sex differences in host susceptibility to infectious agents have prompted numerous studies dealing with the effects of hormones, particularly estrogens, on infectious disease processes[3,4,5]. These studies have clearly indicated that therapeutic, and in some cases physiological, levels of estrogens alter host susceptibility to selected microbial agents[6,7]. Although the underlying modes of action are not completely understood, many potential mechanisms by which estrogens may act to alter the host susceptibility to infection have been identified. One such mechanism identified was modification of host epithelial cell number, morphology, or receptor site synthesis on epithelial cells[4,8]. Other studies have suggested estrogen induced immunosuppression of the immune response. The divergent effects of estrogen on resistance may, in some instances, be due to the quantity of chemical injected or route of infection[9,10]. In addition, it is likely that altered host resistance correlates with immune function disturbances caused by estrogenic compounds. For example, estrogens are potent stimulants of the mononuclear phagocytic system but suppress natural killer cell activity and T cell-mediated immunity as well[11,12,13]. Estrogens may induce these alterations by affecting the production of immunoregulatory thymic hormones[14]. Increased host resistance to certain microorganisms is often attributed to estrogen-related enhancement of macrophage activity[15]. Conversely, estrogen-induced defects in natural killer cell functions and T cell responses may

decrease host resistance to other infectious agents[16]. Other hormone-induced changes in host susceptibility to infection are alterations of host defense factors such as production of cervical mucus in the female genitourinary tract[8].

Receptor proteins have been reported in and are thought to function in an analogous fashion in simple eukaryotes as in higher organisms[17]. Recent studies of the interactions of mammalian steroid hormones with fungi, protozoa, and other microorganisms have demonstrated that hormones may directly alter the virulence of these organisms by various mechanisms including the ability of the organism to adhere to host epithelial cells and by directly altering the growth of the organism[18,19]. In addition, treatment of fungal cultures with steroid hormones has resulted in the demonstration of steroid modulated responses in some pathogenic fungi[20]. It has been postulated that the effect of the hormone may be related to the modulation of the pathogenesis of these fungi[21].

Studies have shown infections with Trichomonas vaginalis are more prevalent after exposure of women to estrogens. In one study the incidence of T. vaginalis infections increased from 4% after one month of oral contraception use to 36% after 3 months and 58% after six months of use. Women practicing other forms of birth control showed no increase in T. vaginalis infections[22]. In an attempt to determine the specific actions and possible pathogenic role of hormones, particularly estrogens, on axenically cultured trichomonads, in vitro growth, chemotaxis, adherence, and hormone binding experiments were

performed with Trichomonas vaginalis (the human female genitourinary tract pathogen) Tritrichomonas foetus (a bovine genitourinary pathogen), and Trichomonas gallinae (a gastrointestinal tract pathogen of birds). 17-β-estradiol was focused on because it is the predominant estrogen found in mammals as well as lower species. The normal human serum level of 17-β-estradiol is 2-3 x 10^{-10} M.

Materials and Methods

Trichomonads

T. vaginalis isolates were cultured from women who were positive for T. vaginalis when saline wet mounts were examined by light microscopy. Sterile cotton swabs were used to inoculate vaginal secretions into 8 x 100 mm borosilicate sterile glass screw-capped tubes containing 6 ml Diamonds DL8 defined medium pH 7.2, supplemented with 8% (v/v) human serum, 40 ug/ml gentamicin and 2.5 ug/ml amphotericin B[23]. Cultures were maintained in DL8 with 4% (v/v) heat-inactivated equine serum at 37°C in air and were subcultured every three to four days (after reaching the stationary phase). Antibiotics were removed from the medium after several passages. Antibiotic-free DL8 medium was used for all Trichomonad experiments. T. gallinae (ATCC #30227 and #30230, American Type Culture Collection, Rockville, MD) were grown in CTLM (ATCC medium formulation #745), washed in PBS, and resuspended in DL8 growth media for all assays. Tritrichomonas foetus isolates were obtained from clinical bovine specimens and were supplied by Dr. Bob BonDurant at the University of California, Davis. These isolates were maintained in ATCC medium #745 and were prepared for assays in a manner similar to the T. gallinae isolates.

Materials [^{3}H]- 17-β-estradiol (100 Ci/mmol) in ethanol was purchased from Research Products International Corp. (Mount Prospect, IL). Non-labelled hormones (Sigma, St. Louis, MO) and the anti-estrogen tamoxifen (Stuart Pharmaceuticals, Wilmington,DE) were dissolved initially in 100% ethanol, and then diluted in PBS, pH 7.3 to yield stock aliquots containing less than 0.1% (v/v) ethanol at 100X final estrogen concentration. Baseline trichomonad medium contained less than 0.1% of the lowest final estrogen concentration used in assays.

Growth Assay. Cultures of trichomonads were centrifuged at 250x g for five minutes and resuspended in 10.0 ml of medium. After vortexing, 0.1 ml (approximately 2.5 x 10^3 organisms) was added to each new tube containing 6 ml of growth medium with varying concentrations of hormones and tamoxifen, an anti-estrogen. The tubes were incubated at 37°C for up to 3 days. Tubes were centrifuged, the supernatant removed, and the trichomonads were resuspended in 1.0 ml PBS and counted with a hemacytometer.

Radiolabelling. Exponential phase isolates (1-2 days) of trichomonads in growth medium were radiolabelled with 5 μ Ci per ml of [^{3}H-] thymidine, specific activity of 78.3 Ci/mmol (DuPont, New England Nuclear Research Products, Boston, MA). Organisms were incubated with label for 18 hours at 37°C and then were washed 3 times in PBS before use in assays.

Chemotaxis. Washed, radiolabelled trichomonads were resuspended in 0.5 ml medium to a concentration of 1 x 10^5 organisms/ml and placed in the bottom of a 24-well transwell plate (Costar, Cambridge, MA). A 0.45 um pore polycarbonate membrane filter insert was placed on top of the trichomonad suspension and 0.5 ml medium was added on top of the membrane. Concentrated estradiol was added to the bottom or the top medium.

After one hour incubation at 37°C during which the migrating trichomonads were trapped in the filters, the membrane inserts were removed, inverted, and blotted to remove excess fluid. The membranes were removed from the inserts and solubilized with 0.5 ml of 0.5M protosol (DuPont, NEN Research Products, Boston, MA). Scintillation fluid was added and radioactivity was assessed in a liquid scintillation counter. Duplicate experiments were done with known quantities of trichomonads to allow conversion of counts per minute (CPM) values to number of trichomonads[24].

Adherence assay. McCoy cells (mouse fibroblasts, not known to respond to estrogens) were cultivated in 24-well Costar culture plates with Eagles minimum essential medium with Earles salts (Gibco labs, Grand Island, NY) plus 10% (v/v) fetal bovine serum (Hyclone Labs, Logan, UT). Medium was removed from confluent monolayers (containing about 2 x 10^5 cells/well) and washed radiolabelled trichomonads, previously incubated for one hour with supplemental estradiol or PBS, were added at a concentration of 2 x 10^6 trichomonads in 1.0 ml medium

consisting of 2 parts cell culture medium and 1 part trichomonad growth medium.

After a 45 minute incubation, non-adherent trichomonads were removed by rinsing each well 3 times with PBS. The contents of each well (McCoy cells and adherent trichomonads) were digested with 0.5 ml 0.1 M NaOH for 30 minutes on a rotator. The digest was pH neutralized with HCl, transferred to scintillation vials containing scintillation fluid, and radioactivity was measured by counting. CPM were converted to the number of trichomonads as described earlier.

Preparation of Cytosol. Trichomonads were cultivated in 100 ml of growth medium to the stationary phase (about 3 days), washed three times in PBS, and resuspended in 5 ml of 10 mM Tris, 1.5 mM EDTA, 250 mM sucrose, 12 mM monothioglycerol, and 10 mM sodium molybdate buffer pH 7.4 at 4°C for 1 hour. Trichomonads were disrupted by sonification in ice by two five second bursts at the lowest setting which microscopically disrupted the cells. After disruption, large cellular fragments were removed by centrifugation at 1,000 x g for 10 minutes at 4°C. This supernatant was ultracentrifuged at 100,000 x g at 4°C for one hour. Cytosol protein concentrations were determined by the Bio-Rad Protein Micro Assay Procedure.

Steroid Binding. To determine total binding, 250 ul aliquots of cytosol were incubated with 6 different concentrations of ^{3}H-17-β estradiol at 4°C for 3 hours (concentrations ranged from $2x10^{-10}M$ to $8x10^{-9}M$). Hormone-receptor binding, which is saturable, was

distinguished from non-saturable, nonspecific binding by performing parallel incubations of radioactive hormone in the presence of a vast excess (200-fold molar excess) of nonradioactive hormone. Such an excess only minimally occupies the nonspecific sites, and therefore does not block nonspecific binding; receptors, on the other hand, are overwhelmingly filled by the excess nonradioactive hormone, and a negligible amount of radioactive hormone binds to them. Bound hormone was separated from free hormone by two ultrafiltrations (by centrifugation) with 10,000 molecular weight cut-off low-protein-binding cellulosic membrane ultrafiltration units (Millipore, Bedford, MA). Retentates were counted in a liquid scintillation counter. Calculated counting efficiency of ^{3}H-estradiol was 32% and background counts averaging 30-40 CPM were subtracted from all calculations. Specific binding was calculated by subtracting non-specific binding from total binding. Standard techniques were used to determine affinity and dissociation constants (Ka,Kd) and binding capacities[17]. Verification of protein in the binding sites was performed by incubation of the cytosol for 20 min with 100 ug/ml trypsin (Sigma) or PBS control at 37°C followed by cooling and performance of the binding assay.

Statistical Methods. All growth, chemotaxis, and adherence experiments were repeated at least 4 times. Estrogen binding experiments were all performed at least three times. Results were analyzed with one- and two- tailed t-tests. Data are expressed as mean $\pm$ standard deviation unless otherwise indicated. Large standard

deviations are due to the day to day variation of the growth of the trichomonads.

Results

Growth. Numerous T. vaginalis isolates were incubated with hormones and the growth of the organisms was measured. Only the estrogens had a significant effect on growth, which was inhibitory. There was no effect of estradiol on the growth of T. foetus or T. gallinae strains when compared to T. vaginalis (Table 1). Growth inhibition did not occur before one day and was maximal after 2-3 days incubation with estrogens at 1-2 ng/ml (3.7-7.4 x 10^{-9}M). The specificity of this effect was verified by its concentration dependency. No effect on growth was noted with concentrations of estradiol less than 1.8x10^{-10}M. Higher concentrations produced no greater inhibition of growth. The specificity was further investigated by using the anti-estrogen, tamoxifen, as an estrogen blocking agent. Tamoxifen alone had no effect on the growth of the T. vaginalis isolates at concentrations of up to 100ng/ml; however, at 1ng/ml it completely blocked the growth inhibition that occurred in the presence of estrogens (Table 2). Exposure to estrogens during growth had no effect on the morphology and motility of trichomonads as observed by 1000x phase-contrast light microscopy. Scanning electron microscopy of glutaraldehyde-fixed trichomonads exposed to 3 days of estradiol also failed to show any morphological effects.

Chemotaxis. The use of a membrane filter separating media with trichomonads on one side, and various media components (i.e. nutrient

Table 1. Effect of 17-β-Estradiol on the growth of trichomonads[#]

	Growth[+]	
Isolate	Control	17-β-Estradiol (1ng/ml)
T. vaginalis-1	1430 ± 701	801 ± 513*
T. vaginalis-2	1481 ± 612	926 ± 523*
T. vaginalis-3	1763 ± 1210	1304 ± 1207*
T. foetus-1	1280 ± 153	1367 ± 361
T. foetus - DI/#3	1146 ± 201	1194 ± 158
T. gallinae - 30227	769 ± 478	761 ± 476
T. gallinae 30230	893 ± 585	797 ± 487

[#] All data expressed as mean ± standard deviation number of Trichomonads x 10^{-3}

[+] Growth after 72 hours

* Significantly different from control ($P<0.05$)

Table 2. Effect of hormones on the growth of T. vaginalis#

	Growth+		
Hormone	Isolate 1	Isolate 2	Isolate 3
None (PBS control)	1402 ± 713	1431 ± 612	1721 ± 1204
17-β-Estradiol (1ng/ml)	800 ± 511*	902 ± 507*	1321 ± 1200*
17-β-Estradiol (1ng/ml and Tamoxifen (10ng/ml)	1521 ± 1120	1431 ± 1001	1624 ± 902
Tamoxifen (10ng/ml)	1362 ± 901	1346 ± 1210	1763 ± 908
Tamoxifen (100ng/ml)	1411 ± 1108	1382 ± 960	1631 ± 942
Estriol (1ng/ml)	913 ± 601*	1101 ± 476	1583 ± 1607
Hydrocortisone (0.4 ug/ml)	1248 ± 1163	1071 ± 928	1638 ± 917
Prednisolone (1ug/ml)	1284 ± 976	1162 ± 714	1638 ± 1701
Progesterone (20ng/ml)	1214 ± 904	1003 ± 713	1648 ± 1528
Testosterone (20ng/ml)	1392 ± 1128	1375 ± 708	1569 ± 1249

All data expressed as mean ± standard deviation number of Trichomonads x 10^{-3}

+ Growth after 72 hours

* Significantly different from control ($P<0.05$)

media) on each side, allowed the assay of chemoattraction as well as chemorepulsion. To assay chemokinesis separately from chemotaxis, the assay was performed eight times with nutrient medium or PBS on both sides of the filter. The chemotactic agent did not affect chemokinesis, as membrane trapping was the same, with a <3% difference whether in PBS or DL8 medium. The chemotaxis of trichomonads towards nutrients was verified by the increased movement and subsequent membrane filter trapping of organisms towards nutrient medium and away from PBS. There were no differences in chemotaxis between trichomonad species towards or away from nutrient media.

Experiments were performed to find the maximum response by independently adjusting incubation times and hormone concentrations. Incubation times ranged from five minutes to 120 minutes and were found to be maximal at 60 minutes, with no additional difference compared to controls after 120 minutes. There was a significant difference in chemotaxis of organisms exposed to estradiol as compared to controls after only 10 minutes, and the difference was quite linear up to 60 minutes, at which time it leveled off but did not decrease. Estradiol concentrations ranged from .001 ng/ml to 1000 ng/ml. The effect was dose dependent between .01 ng/ml and 100 ng/ml with no greater effect seen at higher concentrations. All effects could be blocked with 1 ng/ml tamoxifen; however, tamoxifen alone, at concentrations up to 100 ng/ml, had no effect on the migrating trichomonads. All trichomonads not trapped were motile at the end of experiments (verifiable by light microscopy).

Using this assay system, when supplemental estradiol (10ng/ml) was placed on top of the membrane filter, 15-40% fewer T. vaginalis were trapped in the membrane filter than in the control (chemorepulsion). Similarly, the organisms moved away from their area of containment if supplemental estradiol was present in the bottom well incubation medium, and more were trapped in the membrane filter than for control medium (Table 3). Further, these results are all dose-dependent and occur with estradiol concentrations beginning at .01 ng/ml. Other hormones had no effect on T. vaginalis strains. Two isolates of T. foetus and two of T. gallinae were each found to be totally unresponsive to estradiol with the same assay (Table 3).

Adherence. Initial experiments were performed to find the maximum response by varying incubation times and hormone concentrations. Incubation times were between 15 minutes and 240 minutes and were found to be maximal at 30-45 minutes. There was a significant decrease in adherence of organisms exposed to estradiol after 30 minutes when compared to controls. Estradiol concentrations ranged from .001 ng/ml to 1000 ng/ml. The most significant difference between controls and estradiol exposed trichomonads was seen with 1ng/ml (3.7×10^{-9}M). The effect was dose dependent between .01 ng/ml and 10 ng/ml, with no greater effect seen with higher concentrations.

The adherence of radiolabelled trichomonads to cultured McCoy cells was maximal after 30-45 minute incubation with a ratio of trichomonads to mammalian cells of 10:1. Momentary exposure of the trichomonads to estradiol was not associated with decreased attachment and more

Table 3. Effect of 10ng/ml 17-β-Estradiol on the Chemotaxis of trichomonads[#]

Isolate	Chemotaxis[+] Top (Estradiol above filter)	Bottom (Estradiol below filter)
Control (no Estradiol)	13.8 ± 0.8	13.8 ± 0.8
T. vaginalis-1	9.9 ± 1.3*	20.7 ± 0.9*
T. vaginalis-2	9.7 ± 1.3*	19.3 ± 1.0*
T. vaginalis-3	9.2 ± 1.0*	20.8 ± 0.9*
T. foetus-1	13.3 ± 0.9	13.6 ± 0.7
T. foetus DI/#3	14.0 ± 0.9	13.6 ± 1.1
T. gallinae 30227	13.6 ± 1.0	13.8 ± 0.7
T. gallinae 30230	13.7 ± 1.1	13.6 ± 0.9

[#] All data expressed as mean ± standard deviation number of trichomonads x 10^{-3}

[+] Trichomonads on filter after 45 minute incubation

* Significantly different from control ($P<0.05$)

prolonged (up to 4 hours) incubation of trichomonads with estradiol prior to adherence was not associated with further decreases in attachment compared with 1 hour incubation. Incubation of T. vaginalis strains with estradiol at 1ng/ml (3.7×10^{-9}M) for 1 hour prior to incubation with receptor cells was associated with a statistically significant decrease in adherence to fibroblastic McCoy cells (Table 4). Again, tamoxifen alone, incubated with T. vaginalis at concentrations up to 100ng/ml for 1 hour did not alter subsequent adherence, however, addition of tamoxifen with estradiol in the incubation medium blocked the effects of estradiol. There were no statistically significant differences in adherence to McCoy cells with either T. foetus or T. gallinae after exposure to estradiol (Table 4).

[^{3}H] 17-β-estradiol binding. Protein content of cytosol specimens were as follows: 75.3 ± 7.5 ug/ml from 5.0×10^6 T. vaginalis-1, 85.2 ± 7.3 for T. foetus-1, and 82.9 ± 7.9 for T. gallinae - 30227 (results with other isolates were similar). All isolates displayed specific estrogen binding. The proteinaceous nature of the [^{3}H] 17-β-estradiol binding site in trichomonads was assessed by repeating estrogen binding studies using cytosol incubated with trypsin or PBS control as described in the Methods section. Specific binding after trypsin exposure averaged 8.2 ± 11.0% of control binding for the 5 trichomonad isolates tested. The possibility that binding sites in cytosol preparations were contaminated with residual medium was tested with radiotracer studies. These studies determined that cytosol preparations contained less than 0.00002% medium (v/v) and 0.0001%

Table 4. Effect of 17-β-Estradiol on the Adherence of Trichomonads[#]

	Adherence[+]	
Isolate	Control	17-β-Estradiol (1ng/ml)
T. vaginalis-1	536 ± 41	156 ± 40*
T. vaginalis-2	549 ± 93	213 ± 41*
T. vaginalis-3	433 ± 70	219 ± 52*
T. foetus-1	347 ± 94	349 ± 83
T. foetus - DI/#3	396 ± 92	397 ± 90
T. gallinae - 30227	307 ± 12	298 ± 19
T. gallinae 30230	320 ± 39	321 ± 33

[#] All data expressed as mean ± standard deviation number of Trichomonads x 10^{-3}

[+] Adherent organisms to McCoy cell monolayer after 45 minute incubation

* Significantly different from control ($P<0.05$)

(v/v) of either growth or CTLM medium in cytosol buffer yielded no specific binding of 8 x 10^{-10} M ^{3}H-estradiol.

Estrogen Binding Affinity and Binding Capacity. Scatchard analysis permits the estimation of the number of receptor sites, i.e., specific binding by subtracting nonspecific binding from total binding[17]. This is done by plotting bound steroid (fmole) on the X axis vs. bound/free steroid on the Y axis. The values obtained for specific binding can be used to estimate the number of binding sites and the dissociation constant (K_d) of the receptor-steroid complex. The K_d is equal to the concentration of free hormone at which one-half of the receptor sites are bound. The affinity constant (K_a) is the inverse of Kd or minus the slope (m), (Ka=-m=1/Kd). The theoretical maximal binding is given by the intercept of the line joining the points extrapolated to the X axis[17,18].

The estrogen binding affinity (K_a,K_d) was similar for all isolates; however, there were significant differences in estrogen binding capacity comparing T. vaginalis with the T. gallinae and Tritrichomonas foetus (Table 5). T. vaginalis displayed significantly greater total estrogen binding capacity compared with the non-estrogen responsive trichomonads.

Summary and Conclusions

The attachment of microorganisms to cell surfaces is recognized as the initial step in colonization and subsequent evolution of disease, and bacterial adherence has therefore been closely studied[26]. The host

Table 5. Estrogen binding of trichomonads#

Isolate	Dissociation constant (Kd)+	Binding capacity
T. vaginalis 1	2.9 ± 0.9	40.6 ± 5.3
T. vaginalis 3	3.2 ± 1.3	48.3 ± 9.4
T. foetus -1	2.1 ± 0.9	3.0 ± 0.8*
T. foetus DI #3	2.3 ± 1.2	2.9 ± 0.9*
T. gallinae 30227	3.1 ± 1.1	11.2 ± 6.2*
T. gallinae 30230	3.0 ± 1.7	7.3 ± 3.4*

data expressed at mean ± standard deviation
+ $x10^{-10}$ moles/liters [Kd=1/Ka (affinity constant)]
$x10^{2}$ fmol bound/mg cytosol protein
* $p<0.005$ compared with T. vaginalis-1 using one-tailed t-test

cell-microorganism interaction involves specific receptors sites on host cell surfaces and specific ligands on the microorganisms[27]. The degree of attachment can be determined in vitro by mixing cells and microorganisms under standard conditions and determining the mean number of microorganisms adherent per cell. In vitro adherence of Neisseria gonorrheae, group B Streptococci, E. coli, Proteus mirabilis and Candida albicans to epithelial cells isolated from the female genitourinary tract has been observed[28,29,30]. There have also been reports that the degree of attachment changed with the stages of the menstrual cycle of the cell donors and cyclic changes in adherence were reported for all of these bacteria[28,31]. These data support ideas that hormonal changes may influence bacterial adherence by altering host epithelial cell number and morphology or by modifying receptor site synthesis on epithelial cells. It does not consider direct effects of the hormone on the microorganisms.

In vitro results of hormone acting on trichomonads show that estradiol directly inhibits the growth and adherence of T. vaginalis to mammalian cells and that estradiol acts as a chemorepellant of T. vaginalis. This is another example of how hormones may alter infectivity of microorganisms. It is interesting that trichomonads have the ability to actively move away from a chemorepellant (estradiol) after minutes of exposure while the same chemorepellant takes hours to days longer to effect a demonstratable growth inhibition upon the organisms. Estradiol does not significantly alter these parameters in non-human trichomonads parasites.

Estrogen binding and estrogen initiation of target cell responses have been closely studied. For steroid hormones, receptors are found initially in the soluble intracellular compartment of the cell. After the steroid binds, the receptors modified by the bound hormone, attach to the chromatin of the target tissue cell, and are then found predominantly in the nucleus. Estrogens are lipophilic and therefore may penetrate cell membranes simply by passive diffusion, although specific uptake mechanisms have been postulated[32]. Binding of the hormone by the receptor occurs rapidly inside the cell. This interaction in turn results in a change in the hormone-receptor complex so that the complex then binds to the nuclear chromatin. The binding of receptor-steroid complexes with the nuclear chromatin results in changes in the levels of specific mRNAs. Interestingly, all species of trichomonads retain estrogen binding sites of similar affinity, but T. foetus and T. gallinae, which are not primary pathogens of females have significantly less estrogen binding sites. T. vaginalis isolates, which display physiologic responsiveness to estrogens, have significantly higher estrogen binding capacity than the non-human trichomonads, which do not display physiologic estradiol responsiveness. The binding affinity was similar for all isolates and was quite high. Steroid receptors are expected to have a high affinity for their respective hormones. This is anticipated because the mammalian (or human) blood levels of steroids are usually 10^{-10}M - 10^{-8}M. If a tissue or organism within a host is to respond to a hormone via a receptor mechanism in which the hormone binds to the receptor, then the receptor must have an affinity for the hormone which is in the physiologic range[25,33].

Unfortunately, effects of estrogens on potentially infecting microorganisms may be very different from the effects on the host. It seems that estrogens decrease the infectivity of T. vaginalis in vitro, but the few studies available suggest that estrogens increase infectivity of T. vaginalis in vivo[22,34,35]. Thus, interplay between potentially divergent effects of hormones on microorganisms and the host ultimately determines alterations in infectivity. However, the presence of specific estrogen receptors in T. vaginalis may allow the use of hormonal and antihormonal therapy in the treatment of infections by these organisms.

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