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Diversity of cervicovaginal cytokine response to incident *Chlamydia trachomatis* infection among a prospective cohort of young women

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Abstract

Problem—Animal, *in vitro*, and *ex vivo* studies have identified several cytokines involved in host immunity to genital *Chlamydia trachomatis* (CT) infection. However, *in vivo* cytokine responses are not well-described. Our objectives were to document cervicovaginal cytokine levels and intra-woman cytokine changes during incident CT in a prospective cohort.

Methods—From our prospective cohort, 62 women had incident CT, comprising a CT negative visit followed by a CT positive visit. At these visits, cytokine protein levels (IL-6, IL-8, IL-1α, IL-1β, MIP-1α, RANTES, IFN-γ) were measured using cervicovaginal lavages and the MILLIPLEX™/Luminex® multiplex assay. Quartiles were defined for each cytokine from all 124 visits.

Results—At the group-level, RANTES was higher ($p<0.01$) at the CT positive visit than at baseline, but the other cytokines did not significantly differ. For intra-woman cytokine changes, women with a cytokine level that increased at least one quartile higher (going from baseline to the CT positive visit) ranged between 26–53%. Women with a cytokine level staying in the same quartile ranged between 32–48%. Women with a cytokine level that decreased at least one quartile lower ranged between 15–37%.

Conclusions—Intra-woman cervicovaginal cytokine changes during incident CT appear heterogeneous and may reflect differences in natural host immunity.

Keywords

Adolescent females; Cervicovaginal cytokines; Chlamydia trachomatis; Mucosal immunity

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Introduction

Chlamydia trachomatis (CT) infects the epithelial cells of the genital mucosa and is the most common reportable sexually transmitted infection (STI) in the U.S. The highest rates are consistently found in 15–24-year-old women. The clinical presentation is remarkably heterogeneous, ranging from women with no symptoms, physical findings, or apparent clinical consequences, to those with cervicitis, pelvic inflammatory disease (PID), and/or long-term sequelae. The most serious sequelae include tubal factor infertility, chronic pelvic pain, and ectopic pregnancy, which are typically attributed to PID and the collateral tissue damage from the inflammatory host response.[1] However, host inflammation is often unrecognized, given that inflammation is not consistently reflected by clinical symptoms, the majority of CT-infected women are asymptomatic, and many cases of infertility are understood to be caused by subclinical PID. Ultimately, a better understanding of CT-related inflammatory responses would help to identify women at higher risk for developing sequelae and to inform CT control strategies. Cases with minimal or no apparent symptoms pose a particular challenge, as our understanding of inflammatory mediators in uncomplicated CT infection is especially limited.

Prior knowledge of the host immune response to CT has been largely based on animal, *in vitro*, and *ex vivo* studies or indirectly deduced from epidemiological studies.[2,3] Evidence supports a “cellular paradigm”, in which the CT-infected epithelial cells themselves are the orchestrators of the immune response, releasing cytokines that induce both innate immune cells and adaptive lymphocyte populations. Several inflammatory and regulatory cytokines are identified to be key players. For instance, inflammatory cytokines IL-1α, IL-6, and IL-8 are known to be released by CT-infected host cells in an early and sustained fashion throughout the CT growth cycle,[4,5] and together with inflammatory cytokines IL-1β, TNF, and Th2 cytokine IL-10 these are associated with higher risk for sequelae.[3,6–8] In a study of CT-infected oviduct tissue cultures, IL-1α was the primary cytokine to initiate tissue destruction.[7] In an *ex vivo* study, cervical lymphocytes were collected from CT-infected women and stimulated with CT elementary bodies. Lymphocytes from women reporting a history of fertility disorders were found to secrete higher levels of IL-1β, IL-6, IL-8, and IL-10 compared to those collected from fertile women.[6] In contrast, the IFN-γ producing Th1 phenotype has been consistently demonstrated in the murine model to be required for CT clearance.[3,9] However, few *in vivo* studies of the secretion of these key cytokines during CT infection are available for comparison.[10,11] Although clinical studies of CT-infected women have documented diverse vaginal WBC counts from the similar cervicovaginal compartment where cervical cytokines are secreted,[12,13] the *in vivo* clinical cytokine responses during acute infection are not established. Accordingly, as an initial step to define the *in vivo* cytokine response, our aims were to: 1) document the levels of cytokines found in the cervicovaginal fluid during incident CT among a prospective cohort of young women; and 2) describe intra-woman changes in cytokine levels and explore the possible clinical and behavioral correlates of this intra-woman cytokine change. Given the inherent complexity of host immunity and the heterogeneity of clinical presentation, we hypothesized that women with incident CT demonstrate heterogeneous cytokine responses *in vivo*. We further hypothesized that cytokine levels during infection are associated with another clinical

indicator of inflammation, the elevated vaginal WBC count, but not associated with clinical symptoms given that symptoms can be variable, non-specific, and subject to individual perception.

Materials and Methods

Participants and study design

We selected CT-infected women from an on-going cohort of young women participating in a study of the natural history of human papillomavirus (HPV) previously detailed.[14] Briefly, between October 2000–October 2006, the cohort enrolled 336 women from a family planning clinic, who were 13–21 years old, newly sexually active (5 years maximum), and without history of immunosuppression, cervical neoplasia or cervical procedures. Baseline visits were followed by 4-month interval visits on days without menses, at which the following were collected: clinical interview, colpophotography to document the cervical epithelial appearance, vaginal wet prep microscopy to detect *Trichomonas vaginalis*, Nugents gram stain to detect vaginal WBC count and bacterial vaginosis, and cervical lavage samples to store for future cytokine measurement. Cervical swabs for *C. trachomatis* and *Neisseria gonorrhoeae* were tested at annual visits at a minimum, and additionally at any other visit when patients reported possible STI exposure or genital symptoms. Women were exited from the cohort if they developed immunocompromise or cervical high-grade dysplasia. Each participant gave written voluntary informed consent. The research was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the Committee on Human Subject Research, University of California, San Francisco.

Selected visits

There were 62 (19%) women diagnosed with incident CT infection by commercial nucleic-acid amplification testing of cervical swabs during December 2001–October 2009, who had enough sample for cytokine testing. CT testing was performed using LCx® (Abbott Laboratories) until 2003 and ProbeTec™ (Becton Dickinson) thereafter. The first incident infection during the study was defined as the first episode of a CT positive visit that was preceded by a CT negative visit. We considered this CT negative visit to be the “baseline”. Patients were treated according to guidelines from the Centers for Disease Control and Prevention..

Clinical measures

The following measures were performed by research staff blinded to all patient characteristics including the CT infection status. The vaginal WBC count was evaluated by Nugents gram stain at 1000× magnification (oil-field). WBC ≥5 per high-power field of 400× (equivalent to WBC ≥2 per oil-field) was defined as elevated. CT serology was measured using the micro-immunofluorescence assay applied to blood samples collected before any CT positive detected in the study. Positive serology was defined as a serovar-specified immunoglobulin (IgG) titer ≥1:16, to serve as a marker of prior CT infection. Cervical ectopy, defined as areas of columnar epithelium and early to mid-squamous metaplasia, was quantitatively measured using colpophotography and computerized

planimetry as previously described,[15] because of its influence on cytokine levels.[16] Ectopy was measured at the baseline visit but not at the CT positive visit since cervical inflammation can alter the epithelial appearance. HPV (37 types) was diagnosed by Roche Reverse Line Blot assay applied to cervical lavage samples.

Cytokine measurements

Inflammatory and regulatory cytokines (IL-6, IL-8, IL-1α, IL-1β, MIP-1α, RANTES, IFN-γ, IFN-α2, IL-10, IL-12[p70], TNF-α) were measured in the cervical lavages collected at the selected CT negative and CT positive visits. These cytokines were chosen based on prior work suggesting their roles in CT acquisition, recurrence, and/or sequelae.[3–11] Cervical lavages were collected using a plastic pipette to administer 5cc of sterile normal saline directed just above the cervical os. In order to maximize volume recovery, the fluid was recollected and re-administered for a total of 3 washes over the cervix, then stored at −80°C until testing. After samples were thawed and centrifuged, protein levels of cytokines in the cell-free supernatants were measured (pg/ml) in duplicate using the multiplex MILLIPLEX™ MAP Human Cytokine/Chemokine Kit (Millipore Corp., Billerica, Massachusetts) per manufacturer's instructions. To minimize bias from inter-run variation, all samples from a given woman were run on the same plate, using a Luminex® 100 instrument (Luminex, Austin, Texas). Five-parameter logistic standard curves were fit using MiraiBio MasterPlex QT software (Hitachi Software, South San Francisco, California). The minimum detectable concentrations (pg/ml) defined by the manufacturer were: IL-6=0.9; IL-8=0.4; IL-1α=9.4; IL-1β=0.8; MIP-1α=2.9; RANTES=1.2; IFN-γ=0.8; IFN-α2=2.9, IL-10=1.1; IL-12[p70]=0.6; TNF-α=0.7.

Statistical analyses

This study considered both raw levels of cytokines and intra-woman change from the CT negative visit to the CT positive visit. Individual wells that fell below the minimum detectable concentration were imputed with those minimum values. Since the majority of wells for IFN-α2, IL-10, IL-12, and TNF-α were below detection, these cytokines were excluded from further analyses due to poor assay performance. Samples that were above detectable range were re-run using a 10-fold dilution. One such IL-8 sample was not available for a repeat run and was excluded from IL-8 analyses.

Cytokine levels were not normally distributed and were described using medians and interquartile ranges (IQR). Group-level comparisons between the cytokine levels at the CT negative and positive visits were examined by Wilcoxon rank-sum testing. In order to evaluate consistency of our *in vivo* cytokine measures with another common clinical marker for inflammation (vaginal WBC count), we also used Wilcoxon rank-sum testing for bivariate associations between cytokine levels and vaginal WBCs (≥5/hpf or <5/hpf).

Since normative cytokine levels in cervical lavage samples are not established, we next examined intra-woman change, as we attempted to address inter-women variability by having each woman serve as her own control. We described intra-woman change by defining quartiles for cytokine levels using all 124 visits from the 62 women, and then examining frequencies of a given woman's cytokine level moving from 1 quartile to another

between the CT negative and CT positive visits. This allowed us to describe positive change, no change, or negative change in quartile.

To explore possible correlates of intra-woman cytokine change, we took the following approach. The distributions of cytokine levels were right-skewed. To minimize the bias of estimates due to heteroscedasticity, we used generalized linear regression models with a negative binomial link function and pseudo-maximum likelihood estimator, using a robust variance estimator to adjust for potential overdispersion in the models.[17] The models were fitted with cytokine levels at the CT positive visit as the dependent variables, and included cytokine levels at the CT negative visit in logarithm form as offset terms. Thus the intra-woman changes from the CT negative to positive visits were expressed as cytokine ratios, and we estimated the relative percent differences in cytokine ratios in order to evaluate the influence of the independent variables. The independent variables measured at the CT positive visits included: genital symptoms (any of the following in the past 4 months (yes/no): abnormal discharge, post-coital bleeding, dyspareunia, abdominal pain, pelvic pain, abnormal bleeding); current smoking (yes/no); concurrent HPV infection (positive/negative); concurrent bacterial vaginosis (positive/negative); current oral contraceptive pill use (yes/no); current medroxyprogesterone use (yes/no). Concurrent *N. gonorrhoeae* and *T. Vaginalis* were not analyzed because no cases were diagnosed. The following independent variables were measured only at baseline: CT serology (positive/negative); and amount of cervical ectopy (≥10% or <10% of total cervical face). Significance level for all analyses was set at α=0.05. Statistical analyses were performed using SAS version 9.4 (Cary, NC).

Results

Table 1 shows the distributions of the cytokine levels at the baseline and CT positive visits. RANTES showed higher levels ($p<0.01$) at the CT positive visits compared to baseline, and IFNγ showed a non-significant trend toward higher levels ($p=0.10$), but the other cytokines appeared similar between those 2 timepoints. Cytokine levels at the CT positive visit appear higher among women with higher vaginal WBCs, with some reaching statistical significance (IL-8, $p=0.05$; IL-1α, $p=0.05$; IL-1β, $p<0.01$; IFNγ, $p<0.01$). Similarly, MIP-1α at the baseline visit appeared correlated to vaginal WBC counts as well ($p=0.04$;).

Table 2 shows the distribution of intra-woman change in cytokine level, reported as changes in quartile assignment for the cytokine levels at the baseline and CT positive visits. Frequency of women who had a cytokine level that increased at least one quartile higher (going from baseline to the CT positive visit) ranged between 26–53%; women with a cytokine level staying in the same quartile ranged between 32–48%; and women who had a cytokine level that decrease at least one quartile lower ranged between 15–37%. Only 3 (5%) women exhibited no changes across quartiles for the seven cytokines that we analyzed. The analysis of correlates of intra-woman change is shown in Table 3. Greater intra-woman cytokine change was associated with genital symptoms (IL-1α, $p<0.01$; RANTES, $p=0.02$; IL-8, $p=0.058$). Lesser intra-woman cytokine change was associated with positive CT serology (IL-8, $p<0.01$; IL-1α, $p=0.02$; RANTES, $p=0.02$), medroxyprogesterone use (IL-6, $p<0.01$; IFN-γ, $p<0.01$), and concurrent bacterial vaginosis (RANTES, $p<0.01$). Concurrent HPV infection was associated with greater intra-woman change for IL-1β ($p<0.01$), but

lesser change for RANTES ($p<0.01$). Oral contraceptive pill use had similarly mixed effects, with greater intra-woman change for IL-6 ($p<0.01$) but lesser change for IL-1 α ($p=0.04$),

Discussion

This initial study of women with incident CT infection found that intra-woman cytokine changes appeared diverse during acute infection. For each cytokine, we observed intra-woman patterns that were relatively split between positive change, no change, and negative change across quartiles in cytokine levels. When viewed on the overall group-level, cytokines at the baseline and CT positive visits appeared largely similar to each other, but our alternative assessment of the intra-woman pattern suggests a greater diversity in cytokine response rather than a simple lack of difference. At first glance, diversity in cytokine responses may appear surprising since our participants were all diagnosed with incident infection in a closely followed prospective cohort and did not exhibit a dramatic spectrum of clinical presentation. However, the biological plausibility of our cytokine measurements is supported by the associations between the raw cytokine levels and vaginal WBC count, a common (albeit non-specific) clinical measure from the same cervicovaginal compartment. Additionally, our findings of diverse cytokine responses are consistent with prior studies that found diverse WBC counts during active infection, including lack of elevated WBCs in some cases. One study of 410 CT-infected women at public health clinics found that 46% of women had WBC≤5/hpf on vaginal wet prep,[12] and another study of 29 infected women at an STI clinic found that 35% had WBC<5/oil-field.[13] Alternatively, perhaps the diverse cytokine profiles reflect the variable timing of the specimen collection within the natural course of infection. In a clinical setting, the precise day of exposure and infection is impossible to identify, and an *in vivo* study of CT infection is necessarily observational for obvious ethical reasons.

In our exploration of possible correlates of intra-woman change, genital symptoms were associated with greater intra-woman change for 3 cytokines, which appears plausible if symptoms are reflective of a more inflammatory state. On the other hand, we did not find associations between symptoms and our other 4 cytokines, perhaps because our patients experienced a relatively narrow spectrum of symptoms. For example, other patient populations that exhibit higher incidence of PID may in turn exhibit more consistent associations between symptoms and cytokine responses. Interestingly, we also found that positive CT serology, which serves as a marker of prior infection, was associated with dampened cytokine responses for IL-8, IL-1α, and RANTES. Although non-significant for IL-6, IL-1β, MIP-1α, the model estimates for these cytokines also indicated dampened cytokine responses. These findings prompt the intriguing but speculative question of whether dampened cytokine responses may have contributed to the women's initial vulnerability to the recurrent infection. Further interpretation in this study is not possible given our small sample size with only 6 women testing positive for CT serology. We also found a mixture of associations between the intra-woman change in some cytokines and concurrent HPV, concurrent bacterial vaginosis, oral contraceptive pill use, and medroxyprogesterone use, without a consistent pattern. These findings underscore the importance of including these variables as covariates for adjustment in future *in vivo* studies of cervicovaginal cytokines, but our sample size is not adequate for definitive conclusions

about the direct influence of these factors on cytokine responses to CT infection. Additional influences on cytokine response that should be considered would include host genetic factors, microbial characteristics, or microbial load. Measurements of such factors were not available in our current study but would warrant future investigation.

Common limitations of cytokine studies include the imprecision introduced by instrument and assay variability and sample dilution into saline lavage medium.[18] Given the lack of universally established protocols to measure cervical immune markers, investigators have attempted various approaches including cervical lavage, swab, sponge absorption, and mucus aspiration.19 Our methodology was based on recent work demonstrating the higher recovery of mediators via saline lavage[19] and the intra-laboratory within-run and between-run reproducibility of Luminex.[18–20] Between-run assay variability was further addressed by testing all samples from a given woman on the same plate. It is difficult to further compare our CT-specific findings to available studies of cervical cytokines in non-infected patient populations. We consider our study as an initial step to define cytokine responses in CT-infected women followed prospectively. Our uniquely prospective cohort design allowed each woman's non-infected state to serve as her own normative control. This is a critical advantage when faced with the lack of established normative values and likely inter-woman variation. By focusing only on the group-level cytokine levels, it appears that the levels at the baseline and infected visits were simply similar for all cytokines except RANTES. Consideration of intra-woman changes suggests a possibly more diverse picture. An important limitation is that cervicovaginal cytokines may be influenced by other STIs or unmeasured cervicovaginal microbes. It is difficult to isolate single STIs for study in the clinical setting, given that co-infections are naturally very common. Our study also lacked measurement of serum hormones which vary during menstrual cycles and may influence cytokine secretion. Cervical sample collection could not ethically be deferred for specific menstrual cycle days, as prompt treatment of infection in this clinical setting was necessary. However, keeping these limitations in mind, the overall theme is that CT infection did not demonstrate a dominant cytokine response pattern. Lastly, the long-term clinical significance of cervicovaginal cytokine levels and the clinical significance of the extent of intra-woman change are yet unknown. Our research cohort setting consisted of participants who received frequent screening, made frequent contact with study personnel, and received prompt treatment of any infections. Thus PID was very rare in this setting, and data regarding long-term reproductive sequelae is not available for further analyses.

Conclusions

In summary, we found that *in vivo* cytokine levels during incident CT were similar on the group-level between the baseline and CT positive visits, but intra-woman changes appeared more heterogeneous. Genital symptoms were associated with greater intra-woman change for IL-1α, RANTES, and IL-8. Intra-woman cytokine responses may also be influenced variably by concurrent HPV, concurrent bacterial vaginosis, hormonal contraceptive use, and positive CT serology. If corroborated in larger populations of CT-infected women, this diversity in cytokine responses may reflect differences in natural host immunity and may have implications for risk for sequelae.

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Table 1

Cervicovaginal cytokine levels (pg/ml) before and during incident *Chlamydia trachomatis* infection, and their association with vaginal WBC counts (N=62 women, 124 visits)

	At *Chlamydia* negative visit (baseline, before infection)	At *Chlamydia* positive visit (during infection)		At *Chlamydia* negative visit (baseline, before infection)			At *Chlamydia* positive visit (during infection)		
	Cytokine level, median (IQR)	Cytokine level, median (IQR)	p-value	Cytokine level among women with vaginal WBC ≥5/hpf (n=18), median (IQR)	Median cytokine level among women with vaginal WBC <5/hpf (n=42), median (IQR)	p-value	Cytokine level among women with vaginal WBC ≥5/hpf (n=25), median (IQR)	Cytokine level among women with vaginal WBC <5/hpf (n=37), median (IQR)	p-value
IL-6	30.3 (8.9, 110.6)	30.3 (10.8, 65.6)	0.78	22.2 (11.6, 75.1)	31.0 (8.4, 110.6)	0.80	48.4 (20.5, 78.3)	25.9 (10.3, 60.3)	0.12
IL-8[a]	1951.3 (770.5, 3302.0)	1832.6 (930.5, 2869.3)	0.69	2342.4 (1552.8, 3552.1)	1855.5 (634.8, 3206.7)	0.22	2411.4 (1667.8, 3302.1)	1673.2 (824.1, 2370.9)	0.05
IL-1α	345.3 (150.5, 1077.0)	316.1 (164.8, 867.4)	0.76	446.2 (145.8, 856.8)	335.2 (150.5, 1077.0)	0.90	657.8 (311.6, 1057.9)	240.7 (115.1, 640.0)	0.05
IL-1β	29.0 (5.7, 166.4)	43.3 (11.8, 392.8)	0.17	28.8 (8.8, 108.8)	28.1 (3.7, 219.4)	0.70	251.9 (67.1, 634.2)	13.6 (6.3, 111.3)	<0.01
MIP-1α	14.1 (7.6, 53.8)	19.6 (10.5, 60.3)	0.19	28.2 (17.5, 57.0)	9.8 (6.3, 53.8)	0.04	22.5 (15.1, 68.1)	17.3 (10.2, 53.2)	0.19
RANTES	2.8 (2.3, 5.8)	5.1 (3.3, 19.8)	<0.01	3.3 (2.6, 4.9)	2.7 (2.1, 5.4)	0.28	6.5 (4.2, 10.8)	4.6 (2.6, 21.3)	0.25
IFNγ	0.8 (0.8, 1.4)	1.1 (0.8, 5.2)	0.10	0.9 (0.8, 1.2)	0.8 (0.8, 1.4)	0.78	5.5 (2.3, 12.6)	0.8 (0.8, 2.4)	<0.01

Statistical analyses consisted of Wilcoxon rank-sum tests.

IQR, interquartile range

WBC, white blood cells

hpf, high-power field of 400× magnification

[a]For IL-8, 1 woman was excluded due to assay failure and inadequate remaining sample.

Table 2

Intra-woman change in cervicovaginal cytokine level from the visit before incident *Chlamydia trachomatis* infection to the visit during infection (N=62 women, 124 visits)

	Median (IQR) for all 124 visits	Women with cytokine level that increased at least one quartile higher[a], n (%)	Women with cytokine level that stayed in the same quartile[a], n (%)	Women with cytokine level that decreased at least one quartile lower[a], n (%)
IL-6	30.3 (10.3, 84.3)	17 (28)	25 (40)	20 (32)
IL-8[a]	1869.3 (845.8, 3217.6)	16 (26)	23 (37)	23 (37)
IL-1α	336.5 (157.6, 1019.5)	18 (29)	24 (38)	20 (33)
IL-1β	40.1 (6.8, 289.8)	23 (37)	26 (42)	13 (21)
MIP-1α	18.0 (8.8, 57.0)	25 (40)	22 (36)	15 (24)
RANTES	4.0 (2.4, 10.8)	33 (53)	20 (32)	9 (15)
IFN-γ	1.0 (0.8, 2.9)	23 (37)	30 (48)	9 (15)

IQR, interquartile range

[a]Quartiles for cytokine levels were defined using all 124 visits from the 62 women. Intra-woman change was examined for the cytokine level going from the *Chlamydia* negative visit (before infection) to the *Chlamydia* positive visit (during infection).

[a]For IL-8, 1 woman was excluded due to assay failure and inadequate remaining sample.

Table 3

Associations between the extent of intra-woman change in cervicovaginal cytokine level (from the *Chlamydia* negative to *Chlamydia* positive visit)[a] during incident *Chlamydia trachomatis* infection and clinical and behavioral characteristics among young women (N=62 women, 124 visits)

	n (%)	IL-6 change, Percent-difference (95% CI)	IL-8[b] change, Percent-difference (95% CI)	IL-1α change, Percent-difference (95% CI)	IL-1β change, Percent-difference (95% CI)	MIP-1α change, Percent-difference (95% CI)	RANTES change, Percent-difference (95% CI)	IFN-γ change, Percent-difference (95% CI)
Characteristics measured at the CT positive visit (during infection)								
Had genital symptoms[c] in the last 4 months	27 (44)	31 (−70, 469)	116 (−3, 379)	163** (32, 424)	16 (−83, 689)	−15 (−61, 88)	721* (42, 4639)	54 (−61, 499)
Current smoking	28 (45)	−61 (−87, 13)	−41 (−74, 37)	50 (−41, 285)	−38 (−90, 298)	7 (−51, 134)	−76 (−96, 49)	139 (−34, 770)
Concurrent HPV infection	24 (43)	32 (−70, 480)	15 (−58, 213)	−13 (−63, 104)	918** (163, 3842)	−39 (−71, 27)	−90** (−98, −46)	59 (−60, 523)
Concurrent bacterial vaginosis	13 (21)	−65 (−88, 7)	−35 (−73, 57)	−7 (−60, 115)	120 (−66, 1327)	70 (−32, 325)	−89** (−98, −49)	184 (−34, 1121)
Current oral contraceptive pill use	12 (19)	581** (126, 1956)	−18 (−64, 90)	−60* (−83, −6)	210 (−60, 2315)	26 (−56, 263)	907 (47, 6815)	−27 (−87, 300)
Current medroxyprogesterone use	9 (15)	−65** (−83, −30)	27 (−74, 518)	−50 (−82, 37)	−66 (−92, 44)	−40 (−69, 15)	−52 (−83, 34)	−86** (−96, −42)
Characteristics measured at the CT negative visit (baseline, before infection)								
Positive CT serology[d]	6 (15)	−58 (−88, 47)	−67** (−83, −37)	−59* (−81, −14)	−76 (−95, 9)	−27 (−69, 70)	−89* (−98, −34)	−76 (−94, 5)
Cervical ectopy >10% of the total cervical face[e]	23 (43)	−55 (−87, 51)	61 (−44, 360)	36 (−48,256)	−83* (−96, −28)	−21 (−67, 87)	−82 (−97, 17)	−43 (−85, 125)

* p<0.05,

** p<0.01

CI, confidence interval

[a] Negative binomial regression models were constructed to examine the relative percent difference in cytokine ratio (cytokine level at the *Chlamydia* positive visit / cytokine level at the *Chlamydia* negative visit) associated with each independent variable.

[b] For IL-8, 1 woman was excluded due to assay failure and inadequate sample.

[c] Genital symptoms were defined as any of the following: abnormal discharge, post-coital bleeding, dyspareunia, abdominal pain, pelvic pain, abnormal bleeding.

[d] For *Chlamydia* serology testing, samples were available for 41 women.

[e] Cervical ectopy was defined as areas of columnar and early-mid squamous metaplasia and quantitatively measured as a percentage of the total cervical face. Colpophotographs were available for 53 women.