Susceptibility of Middle Adolescent Females to Sexually Transmitted infections: Impact of Hormone Contraception and Sexual Behaviors on Vaginal Immunity

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Keywords
Adolescent behaviors, adolescents, sexually transmitted infections, vaginal immunity

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Submitted December 20, 2006;
accepted May 9, 2007.

Citation
Barousse MM, Theall KP, Van Der Pol B, Fortenberry JD, Orr DP, Fidel PL, Jr. Susceptibility of middle adolescent females to sexually transmitted infections: Impact of hormone contraception and sexual behaviors on vaginal immunity. Am J Reprod Immunol 2007; 58:159–168

doi:10.1111/j.1600-0897.2007.00504.x

Problem
The resistance and/or susceptibility to infections can be influenced by patterns of immunomodulators. Based on this and the high rate of sexually transmitted infections (STIs) in adolescents, we examined the longitudinal relationship between sexual behaviors, hormonal contraceptive use, and bacterial vaginosis (BV) with vaginal-associated immunomodulators in adolescent females.

Method of study
Over 27 months, subjects completed detailed questionnaires, and consented to vaginal swabs for STI testing, and vaginal lavages for identification of immunomodulators including T-helper, proinflammatory, and chemokines. Concentrations of immunomodulators were correlated with each parameter together with prevalence of STIs.

Results
Each parameter had a limited influence on vaginal immunomodulators with no evidence of any pattern(s) associated with infection. Conversely, the local presence of proinflammatory cytokines and neutrophils in those with an STI indicated some immune responsiveness.

Conclusion
Sexual behaviors, contraceptive usage, and BV do not appear as factors in susceptibility of adolescents to STIs through the influence of local immunomodulators.

Introduction

Adolescent girls represent a menarchal group of females that is newly influenced by reproductive hormones and are also extremely susceptible to sexually transmitted infections (STIs); including but not limited to *Chlamydia trachomatis* (CT), *Trichomonas vaginalis* (TV), and *Neisseria gonorrhoeae* (GC). An estimated 3 million adolescents acquire an STI annually in the United States.[1] Although vaginal immunity and STIs have been studied frequently in adult women, [2–6] these parameters have been understudied in adolescent females.

Mucosal immunity has been considered the major contributing factor to host defense in the female reproductive tract and has been described as being independent from the systemic system.[7] Cervical vaginal secretions have been shown to contain

detectable levels of immunomodulators including Th1-/Th2-type and proinflammatory cytokines, chemokines, and immunoglobulins that all play some role against invading pathogens, including those that cause STIs. Th1-type responses are responsible for phagocyte-mediated defense against intracellular microorganisms, whereas Th2-type responses are involved in the resistance to extracellular pathogens through predominantly antibody production. Proinflammatory cytokines play a role in acute inflammation, whereas chemokines induce cell migration.[8] Local factors, including vaginal epithelial cells, have also been shown to contribute to the immune defense in the vagina.[5,9] Other cellular components including cytotoxic T cells, natural killer cells, macrophages, and granulocyte-mediated defense mechanisms are important in host defense against pathogenic bacteria and viruses. Changes or induced presence of specific cytokines or lack thereof in these immunodulators by any number of behaviors, conditions, or substance usage could influence susceptibility to STIs. For example, the presence of Th2-type cytokines might enhance susceptibility to GC or CT, whereas Th1-type cytokines might enhance susceptibility to TV. The lack of proinflammatory cytokines might enhance susceptibility to any of the aforementioned infections.

In a previous cross-sectional study examining this population of adolescents at enrollment it was observed that the rate of STIs was 18% and predominated by *Chlamydia*, while the rate of vulvovaginal candidiasis (VVC) was very low at 2%.[10] The rate of bacterial vaginosis (BV) was 36%. [11] Immunological properties included a Th2-type vaginal cytokine profile and vaginal antibodies dominated by IgA.[10]

The purpose of the present study was to examine the study population longitudinally for the impact of hormonal contraception, sexual behaviors, and BV on vaginal immunity as potential factors associated with susceptibility to STIs.

Materials and methods

Participants/Specimens Collected

Between May 1999 and December 2003, 231 middle adolescent females (ages ranging 14–17) were enrolled as part of the Mid-America Adolescent Sexually Transmitted Disease Clinical Young Women's Project based in Indianapolis, IN. Accrual was continual (dynamic cohort) and visits took place every 3 months for 27 months. The number of visits ranged from one (baseline only) to ten (all visits completed), with 32 respondents (14.7%) having only a baseline visit and 75 (32.5%) having all ten visits. The total number of visits that these 231 patients made over the 3 year follow-up was 1614. Enrollment was based on attendance to an adolescent health clinic for multiple reasons (periodic health care, oral contraception, gynecological symptoms, pregnancy testing, attendance with a friend, etc). Having a STI or symptoms of a vaginal condition was not a prerequisite.

Informed consent was obtained from all participants as well as permission from the accompanying parent/guardian for entry into the study. All procedures were followed in the conduct of clinical research in accordance with the Institutional Review Boards at Indiana University School of Medicine (IU), Indianapolis, IN, and Louisiana State University Health Sciences Center (LSUHSC), New Orleans, LA. At IU, participants were asked to complete a detailed questionnaire including information on sexual behaviors, contraceptive usage, and demographics at time of enrollment and at each subsequent visit. Specimens were also collected at the time of enrollment and at each subsequent visit. Specimens collected at IU were shipped overnight to LSUHSC where they were processed and analyzed.

Specimens collected at the 3 month intervals from enrolled subjects included vaginal smears, vaginal swab, endocervical swab, vaginal lavage, and blood (10 mL) by venipuncture into red top tubes (without additives) (Becton Dickinson, Sparks, MD, USA). A provider collected all specimens at scheduled visits. Following the collection of the vaginal swab for smears and culture, the vaginal lavage was collected after a 30–40 s aspiration with 5 mL of non-pyrogenic sterile saline. An endocervical brush was gently used prior to the lavage in order to facilitate the release of sloughing vaginal epithelial cells for adequate numbers in epithelial cell testing. The lavage fluid was processed as previously described [10] and stored at −70°C until use. The lavage cell pellet was stored at −70°C in cryopreservative medium [50% fetal bovine serum (FBS), 30% RPMI 1640 medium, and 20% DMSO; GIBCO, Grand Island, NY, USA] until use. One vaginal smear was spray fixed and stained using Papanicolaou-staining technique. The second vaginal smear was gram stained. Serum was collected from clotted blood following centrifugation and stored at −70°C until use.

STI Testing

The presence of CT, TV, and GC was examined for each patient at each visit using polymerase chain reaction (PCR) using the COBAS AMPLICOR CT/NG test and a modification of this assay to test for TV from vaginal swabs [12,13] (B. Van Der Pol, J.A. Williams, N.J. Smith, and R.B. Jones, Abstr. 4th European Chlamydia Meeting, 2000.)

Behavioral Characteristics

In the questionnaire, participants provided information on current sexual behaviors, including number of sex partners, frequency of coitus, and condom usage. The number of sexual partners within the previous 3 months and never having coitus or not having coitus within the 3 months prior to the interview was also provided. Participants also provided the date of their last coital event. Influences of coitus in the last week and in the last month were examined based on that date and the corresponding date of the interview. Consistent condom use in the 3 months prior to the interview was based on the reported number of coital events occurred with each partner and the number of those times that a condom was used, calculated as a percentage. One hundred percent was considered consistent use. Douching patterns were also examined and included current douching (yes/no) and the frequency of douching. Participants provided the date of their last douche and douching in the last week and last month were examined based on the date and the corresponding interview date.

Identification of the Menstrual Cycle

Stage of the menstrual cycle was verified by estradiol and progesterone concentrations in sera by radioimmunoassay (RIA) at the clinical endocrinology laboratory in the division of reproductive endocrinology at the Detroit Medical Center, Detroit, MI, USA.

Identification of Bacterial Vaginosis

The gram stained vaginal smear was evaluated for bacterial flora. The flora was scored using Nugent's criteria (0–3: normal; 4–6: intermediate, and 7–10: BV).[14]

Scoring of Polymorphonuclear Neutrophils

The vaginal smear stained using Papanicolaou-staining technique was evaluated for evidence of polymorphonuclear neutrophils (PMNs). A scoring system of 1–11 was used to quantify the neutrophils and defined as 1 = 1–10 PMNs/5 fields, 2 = 11–20 PMNs/5 fields, 3 = 21–30 PMNs/5 fields, and continuing with a similar pattern up to 11 = >100 PMNs/5 fields.

Immunomodulators

For each available sample, cervicovaginal lavage fluid (CVL) was evaluated for Th-type and proinflammatory cytokines, and chemokines. The Th1-type cytokines included interleukin (IL)-2, interferon (IFN)-γ, and IL-12. The Th2-type cytokines included IL-4, transforming growth factor (TGF)-β, and IL-10. The proinflammatory cytokines included IL-1α, IL-6, and tumor necrosis factor (TNF)-α. The chemokines included IL-8, RANTES, and macrophage chemoattractant protein (MCP)-1.

Cytokines and chemokines were quantified by capture ELISA (BD Pharmingen, San Diego, CA, USA) according to manufacturer's instructions and as previously described.[15,16] Absorbance was read at 450 nm using an automated plate reader (Bio-Tek Instruments, Winooski, VT, USA). Concentrations for each sample were extrapolated from the standard curve and expressed as pg/mL. The total protein content was determined for each vaginal lavage sample using the BCA protein assay kit (Sigma-Aldrich, St Louis, MO, USA) as previously described and expressed as mg/mL.[10] All cytokine and chemokine concentrations were ultimately normalized to total protein in the sample and expressed as pg/mg protein.

Statistical Analysis

Univariate, bivariate, and multivariate methods were employed to analyze data. All analyses were performed with SAS version 9 (SAS Institute, Cary, NC, USA). Bivariate analyses included Pearson's or Spearman's correlation coefficients and one-way ANOVA or Mann–Whitney *U*-test, where appropriate. The crude correlation between demographic, behavioral, and clinical characteristics and each immunomodulator were examined at baseline and over all visits (i.e. all time points) to determine which factors

were significantly or marginally ($P < 0.10$) associated with each immunomodulator. Factors associated with each immunomodulator were then examined multivariately. Multivariate linear regression was used to examine differences between behavioral and clinical characteristics and each immunomodulator, while controlling for demographics, the presence of BV [known to influence immunomodulators][11], and other behavioral characteristics. One regression model per immunomodulator was examined, with the immunomodulator as the dependent variable. Values of the immunomodulators were log-transformed to normalize the distribution where needed. Regression models were run using SAS's PROC MIXED for repeated measures, examining changes in immunomodulators according to characteristics over time and changes in immunomodulator levels over time (using time as a covariate in the model and controlling for the baseline level).

Repeated measures analysis in this framework allows for intra-subject correlation among repeated measurements from the same subject – accounting for correlation and dependent error terms due to repeated observations.[17] The method also weighs responses according to the number of visits/waves per unit of analysis (adolescent). Generalized estimating equations (GEE) were also used for comparisons and results were identical. Both methods take into account the correlation between responses over time. Results are reported as statistically significant ($\alpha = 0.05$) or as marginally significant ($\alpha = 0.10$) trends. All data used for comparisons were reflective of the condition at the time point the vaginal lavage was taken unless stated otherwise.

Results

Demographics, Behavioral, and Clinical Characteristics and Sexually Transmitted Infections

Table I presents baseline and overall demographics, and behavioral and clinical characteristics of participants ($n = 231$ with visits ranging from 1 to 10 over the 2.5-year period) including age and race of participants, BV prevalence, endogenous hormone concentrations, hormonal contraceptive usage, sexual activity and condom usage, douching behaviors, and STI prevalence. There were few differences between parameters over time, with the exception of an increase in recent (past week) coitus ($\chi^2 = 19.63$, $P < 0.0001$).

Table I Characteristics of Participants at Baseline/Enrollment (N = 231) and total visits (N = 1614)

	Enrollment *n* (%)	Total visits *n* (%)
Age category (years)		
14	57 (24.7)	–
15	75 (32.5)	–
16	67 (29.0)	–
17	32 (13.9)	–
Mean age (±SD)	15 (±1.0)	–
Race		
African American	203 (87.9)	–
Caucasian	29 (12.6)	–
Hispanic ethnicity (yes)	3 (1.3)	–
Bacterial vaginosis positive	21 (25.3)	174 (10.8)
Endogenous hormone levels		
Median estrogen ng/mL (range)	43.2 (5–569.7)	41.2 (1.2–20833.0)
Median progesterone ng/mL (range)	0.61 (0.21–21.7)	0.60 (0.10–103.9)
Hormonal contraceptive use		
Any contraception	125 (54.1)	816 (50.6)
Oral contraceptive	51 (24.5)	289 (18.60)
Depo-Provera	76 (37.6)	531 (34.7)
Any sexually transmitted infection (yes)	43 (18.6)	286 (17.7)
Chlamydia trachomatis (yes)	26 (11.4)	167 (10.4)
Neisseria gonorrhea (yes)	12 (5.3)	97 (6.0)
Trichomonas vaginalis (yes)	13 (5.7)	93 (5.8)
Co-infection with 2 or more (yes)	8 (3.5)	67 (4.2)
Douches (yes)	76 (33.5)	409 (25.4)
Douched in last week (yes)	16 (6.9)	100 (6.2)
Sexually active (yes)	172 (78.9)	1372 (86.5)
Sex in the last week (yes)	51 (22.1)	559 (34.6)
Consistent condom use (100%) with partner(s) (yes)	43 (49.4)	483 (45.8)
Used condom during last vaginal sex (yes)	58 (59.8)	610 (54.1)

Note: Totals may not add up to N due to missing data; *n* (%) represents estimates of those with non-missing responses.

Over time, the period prevalence of BV was 54.4% and the period prevalence of participants on hormonal contraception at least once during the follow-up period was 76.6% [46.1% using an estrogen-dominant- based form (oral contraceptives) and 51.8% using a progesterone-dominant-based form (Depo-Provera) (Pfizer, New York, NY, USA)]. Also, over time, 50.2% of respondents reported douching,

with 19.1% of respondents reporting douching (at least once) in the week prior to the interview.

At enrollment, 53.2% reported coital activity in the 3 months prior to the interview and specimen collection and 21.1% (n = 46) reported never having coitus. The proportion of participants reporting recent (previous 3 months) coital activity over time was 80.4% of subjects with 63.2% having coitus in the previous week. Increased age was significantly associated with an increased likelihood of coitus over the course of the study (χ^2 = 21.27, $P < 0.0001$).

In addition to general STI prevalence (Table I), a few participants had co-infections. These included two positive for CT and TV, three positive for CT and GC, and three positive for GC and TV. Nearly half of respondents (n = 111, 48.1%) tested positive for at least one STI pathogen during the course of the study period, 84 (36.4%) with CT, 46 (19.9%) with GC, and 41 (17.7%) with TV.

Local Immunomodulators

Local immunomodulator levels at baseline/enrollment and over all time points (average over time) are presented in Table II. At baseline Th1- (IL-2, IL-12, IFN-γ) and Th2- (IL-4, IL-10, TGF-β) type cytokines were detectable (above the sensitivity of the assay) in ~75% of available lavage samples with concentrations of Th1-type cytokines dominated by IL-2 and concentrations of Th2-type cytokines dominated by IL-4. Proinflammatory cytokines (IL-6, IL-1α, TNF-α) were detectable in all available lavage samples tested and were dominated by IL-1α. Chemokines (IL-8, RANTES, MCP-1) were detectable in ~73% of the available lavage samples and dominated by IL-8. The percent of samples that had detectable levels of cytokines and chemokines longitudinally were similar to that of enrollment. The Th2 dominating profile at baseline was also dominant at endpoint although TGF-β significantly decreased at endpoint. Proinflammatory cytokines and chemokines continued to be dominated by IL-1α and IL-8, respectively.

Changes in the distribution of vaginal-associated Th1, Th2, and proinflammatory cytokines and chemokines over time were examined with repeated measures using time as the independent variable of interest. Standardized beta estimates, confidence intervals, and t-test statistics based on these changes are also presented in Table II. For Th1 cytokines, concentrations of IFN-γ and IL-12 were fairly similar over time, while concentrations of IL-2 declined significantly. For Th2 cytokines, concentrations of IL-4, IL-10 and TGF-β were all significantly variable over time. For proinflammatory cytokines, only concentrations of IL-1α declined significantly over time. For chemokines, concentrations of IL-8 and MCP-1 were significantly variable overtime.

Table II Median Level of Vaginal Immunomodulators, pg/mg Protein (range), and Variability Over Time

Immunomodulator (n at enrollment/baseline)	Enrollment Median (range)	Total Visits Median (range)	Standardized beta (confidence interval)	t-test statistic for change over time
IL-2 (n = 98)	140.7 (0–3554.6)	76.7 (0–3554.6)	−0.2157 (−0.3085 to −0.1214)	−4.52‡
IL-12 (n = 118)	51.1 (0–1779.5)	35.8 (0–8300.0)	0.0398 (−0.0522 to 0.1318)	0.85
IFN-γ (n = 122)	24.8 (0–2502.1)	6.7 (0–16470.5)	0.1212 (−0.0950 to 0.3374)	1.10
IL-4 (n = 117)	86.6 (0–12314.8)	23.8 (0–12314.8)	−0.3426 (−0.6038 to −0.0814)	−2.58‡
IL-10 (n = 117)	0 (0–4043.4)	4.5 (0–4935.0)	−0.0598 (−0.0787 to −0.0408)	−6.20‡
TGF-β (n = 65)	429.5 (63.5–4123.3)	325.7 (0–15966.4)	−0.1901 (−0.2381 to −0.1421)	−3.76‡
IL-1α (n = 104)	775.0 (36.4–15731.4)	812.0 (6.9–15731.4)	−0.0206 (−0.0281 to −0.0131)	−5.40‡
IL-6 (n = 110)	355.5 (31.3–5308.1)	326.3 (0–12456.4)	−0.3817 (−0.8565 to 0.0931)	−1.58
IL-8 (n = 109)	622.2 (0–14970.6)	495.5 (0–30405.7)	−0.3852 (−0.5148 to −0.2556)	−5.83‡
RANTES (n = 59)	17.6 (0–2247.6)	21.9 (0–4968.6)	−0.0610 (−0.2164 to 0.0943)	−0.77
MCP-1 (n = 49)	111.1 (0–1715.3)	312.2 (0–99239.4)	−0.3674 (−0.7236 to −0.0112)	−2.02‡
TNF-α (n = 107)	58.9 (2.6–6891.4)	33.9 (0–6891.4)	0.5076 (−0.3080 to 1.3231)	1.23

Note: Totals may not add up to N due to missing data; n (%) represents estimates of those with non-missing responses.
IL, interleukin; TGF-β, transforming growth factor-β.
‡P-value < 0.05, based on repeated measures (with GLMM) beta estimates and t-test statistic.

Associations of Behaviors and Clinical Parameters with Local Immunomodulators

Table III presents the crude correlation between immunomodulators in vaginal lavage fluid and select behavioral and clinical parameters, while Table IV presents results from select multivariate linear regression models. Multivariate linear regression models were run for all immunomodulators, but due to space limitations we present only those for IL-1α, IL-8, IL-10, IL-12, TGF-β, and TNF-α. With respect to demographic characteristics, concentrations of IL-8, IL-10, IFN-γ, and IL-12 declined with age. Hispanic ethnicity was also inversely associated with concentrations IL-10, IL-12, and TGF-β and these associations remained in the multivariate models.

Impact of BV on local immunomodulators

Among adolescents with BV, significantly greater concentrations of IL-1α, IL-8, and TGF-β were

Table III Crude Spearman or Pearson's Correlation (Rho) Between Immunomodulators and Selected Characteristics (over all time points)

	Immunomodulator											
	IL-2	IL-12	IFN-γ	IL-4	IL-10	TGF-β	IL-1α	IL-6	IL-8	RANTES	MCP-1	TNF-α
Mean age (±SD)	−0.079	**−0.095**‡	**−0.078**†	−0.039	**−0.131**‡	0.089	−0.010	−0.043	**−0.073**†	0.009	−0.067	−0.047
African American race (yes)	0.034	0.054	0.010	−0.038	0.014	0.083	0.032	0.044	0.021	0.049	0.084	0.022
Hispanic ethnicity (yes)	0.002	**−0.050**†	−0.003	−0.003	**−0.040**†	**−0.023**†	−0.063	0.012	0.081	−0.033	−0.021	0.008
Bacterial Vaginosis positive	0.037	0.008	−0.009	0.010	−0.014	**0.211**‡	**0.018**‡	0.034	**0.183**‡	−0.042	−0.016	−0.026
Hormonal contraceptive use												
Oral contraceptive	−0.027	−0.045	−0.013	0.019	0.018	**−0.061**†	−0.021	0.046	−0.011	**0.094**†	−0.005	−0.038
Depo-Provera	**0.085**†	**0.037**†	0.032	**0.079**†	**0.038**†	**−0.112**‡	−0.008	−0.013	0.025	0.039	0.054	**0.078**†
Any sexually transmitted infection (yes)	−0.002	**−0.066**†	−0.023	−0.016	0.006	**0.024**†	**0.100**‡	**0.063**†	**0.048**‡	−0.047	−0.002	0.035
Douched in last week (yes)	**0.110**‡	−0.038	−0.002	**0.139**‡	**−0.103**‡	**0.215**‡	**0.123**‡	0.068	−0.021	**0.128**‡	−0.013	**0.175**‡
Sex in the last week (yes)	**−0.083**†	−0.048	**−0.062**†	**−0.089**†	**−0.082**‡	−0.065	**−0.094**†	**0.046**†	−0.001	0.007	**−0.106**‡	−0.054
Consistent condom use (100%) (yes)	−0.017	**0.091**†	0.048	−0.056	−0.014	0.028	0.019	**0.149**‡	−0.043	**−0.076**†	0.072	−0.003

Note: *P*-value (two-sided): $^{\dagger}P < 0.1$; $^{\ddagger}P < 0.05$.
IL, interleukin; TGF-β, transforming growth factor-β.

Table IV Factors Associated with Select Immunomodulators (pg/mg protein) Over Time – Beta Estimates from GLMM Multivariate Regression Models (N = 231 adolescents, N = 1614 visits)

Variables	IL-12	IL-10	TGF-β	IL-1α	IL-8
β (constant/intercept)	**−3867.90**‡	−903.64	**34560.26**‡	−17347.50	−20027.30
Time	0.27	0.05	**−2.53**‡	1.45	1.65
Age (years)	0.07	8.53	218.72	−164.06	−242.24
African American (yes)	−72.38	0.65	−203.16	−125.63	−70.03
Hispanic ethnicity (yes)	**−171.74**‡	**−52.91**‡	**−391.43**†	−276.89	1257.64
BV (yes)	−53.61	6.81	**306.32**†	**866.22**‡	**671.10**‡
Oral contraceptives (yes)	−53.60	29.06	**−449.08**‡	−373.48	369.15
Depo-Provera (yes)	−9.60	−4.61	34.19	−326.74	78.24
Any STI (yes)	−7.95	−2.37	**262.09**‡	234.67	**378.34**‡
Douched in the last week (yes)	−33.96	35.21	−55.01	−886.89	−69.88
Sex in the last week (yes)	−5.90	−2.21	−48.38	−167.91	133.53
Consistent condom use (100%) (yes)	**59.74**‡	4.49	254.13	65.04	−116.54

Note. *P*-value (two-sided): $^{\dagger}P < 0.1$; $^{\ddagger}P < 0.05$.
IL, interleukin; TGF-β, transforming growth factor-β; BV, bacterial vaginosis; STI, sexually transmitted infections.

observed at enrollment (Table III) and remained significant ($P < 0.05$) over time and after controlling for other covariates (Table IV).

Impact of hormonal contraceptive use on immunomodulators

Oral contraceptive use was associated with increased concentrations of RANTES but decreased concentrations of TGF-β. Depo-Provera use was associated with increased concentration of IL-2, IL-4, IL-10, IL-12, and TNF-α and decreased concentrations of TGF-β (Table III). The association between oral contraceptive use and concentrations of RANTES (data not shown) and TGF-β (Table IV) remained in multivariate models. None of the observed differences in immunomodulators from Depo-Provera use remained in multivariate models. Endogenous hormone levels showed no associations with immunomodulators at baseline or over time (data not shown).

Impact of douching and sexual behaviors on immunomodulators

Having douched in the week prior to the interview was associated with increased concentrations of IL-1α, IL-2, IL-4, RANTES, TGF-β, and TNF-α and decreased concentrations of IL-10 (Table III). Differences in immunomodulators according to recent douching remained in multivariate models only for concentrations of IL-2 and MCP-1 (data not shown).

Sexual activity had significant influences on immunomodulator concentrations. Recent (last week) sexual activity was inversely associated with concentrations of IFN-γ, IL-1α, IL-2, IL-4, IL-10, and MCP-1 and positively associated with concentrations of IL-6 (Table III). Consistent condom use for vaginal sex was also positively associated with concentrations of IL-6, as well as IL-12, and inversely associated with RANTES. Recent sexual activity remained significantly associated with concentrations of IL-6 and MCP-1 in multivariate models (data not shown) along with consistent condom use and concentrations of IL-6 and IL-12 (data not shown).

STIs and immunomodulators

Greater concentrations of IL-1α, IL-6, IL-8, and TGF-β and lower concentrations of IL-12 were observed among adolescents with an STI (Table III). Observed differences in concentrations of IL-8 and TGF-β remained even after controlling for other covariates (Table IV). With respect to specific STIs, participants with GC presented with significantly ($P < 0.05$) higher concentrations of IL-1α compared to their uninfected counterparts. Those with CT had significantly higher concentrations of IL-8, while participants with TV had significantly higher concentrations of IL-1α, IL-8, and TGF-β (data not shown).

Polymorphonuclear neutrophil scores in vaginal smears were quantified for 183 subjects longitudinally. Subjects that were positive for any STI pathogen had a significantly higher mean PMN score (2.4 ± 0.3) than those not infected with an STI (1.8 ± 0.1) ($P = 0.005$). Specifically, subjects infected with TV (3.6 ± 0.8) or subjects with a co-infection including TV (3.2 ± 0.8) had a significantly higher mean PMN score than those not infected with any STI ($P = 0.0002$ and 0.004, respectively).

Discussion

The objective of the present study was to examine the impact of contraceptive use, sexual and douching behaviors, and BV on vaginal immunomodulators as a means to identify potential factors in susceptibility of adolescents to STIs. Characteristics at enrollment remained similar over the course of the study, with the exception of increased recent coitus.

Overall, the local cytokine patterns of this adolescent population were consistent over time. For example, concentrations of proinflammatory cytokines were greater than other immunomodulators throughout the study. However, concentrations of several specific immunomodulators varied over time including: IL-1α, IL-2, IL-4, IL-8, IL-10, TGF-β, and MCP-1.

While the presence of BV had an impact on immunomodulators, there was no pattern to those affected (TGF-β – anti-inflammatory, IL-12 – proinflammatory, IL-8 – chemokine) at enrollment or over time. Interestingly, however, these are the same cytokines that were affected by the presence of TV. A study by Moodley et al. showed that TV is strongly associated with an abnormal vaginal ecology [18] suggesting that TV may contribute to the development of BV with the cytokines manifestations caused by TV or BV. Interestingly, we found a positive association between TV and BV ($P = 0.0013$) in the present study.

Although endogenous hormones were considerably low (analogous to a follicular condition) and had no impact on the parameter of vaginal immunity, exogenous hormones were associated with some fluctuation of immunomodulators. The use of

Depo-Provera was associated with several increases in cytokines compared to oral contraceptives. However, few observed differences remained consistently associated with immunomodulators over time with the exception of oral contraceptive use and local RANTES and TGF-β concentrations. Interestingly, Franklin and Kutteh have shown an association on oral contraceptive usage with increased antibodies (IgA, IgG), and IL-1β in cervical mucus, [19] and reproductive hormones in general have been associated with reduced immune reactivity.[20] This was not evident, however, in this population. Thus, the precise relationship between levels of local immunomodulators and contraceptive use remains unclear.

As sexual activity is quite prevalent in the adolescent population [21], it was important to examine the influences of sexual activity and other sexual behaviors on local immunomodulators. Although a number of changes in immunomodulators were observed with certain sexual behaviors and douching patterns, there was no specific pattern of changes that was suggestive of a relationship between the behaviors and the immunomodulators or any impact of those changes on susceptibility to STIs. To our knowledge no other studies have analyzed the impact of these behaviors on local immunomodulators, including adults and adolescents, although several studies have shown the predictive value of these behaviors for STIs and HIV infection/transmission.[22–24] Hence the results of this study do not support a role for sexual behaviors, hormonal contraception, or BV in susceptibility of adolescents to STIs through the influence on vaginal immunomodulators that could create a microenvironment (pattern of cytokines) conducive to infection. We recognize, however, that each parameter may play a role in susceptibility to STIs through other means or that the presence of individual cytokines that can be associated with susceptibility to infections do indeed play a role locally despite the simultaneous presence of those that would counter their actions.

We recently reported that, at enrollment, 18% of this cohort had a STI.[10] This prevalence remained unchanged in the longitudinal analysis although the prevalence over the 27-month period showed that 48% of subjects had a STI at some time during the observation period. While the prevalence of STIs was high in this adolescent population, the prevalence and distribution of particular STIs were consistent with national statistics[1] and expected given the nature of recruitment (adolescent health clinic). In this population, infection with CT was highest (11.4%). This is consistent with other reports showing CT as being the most prevalent STI among adolescents.[25] Levels of GC and TV were also similar to published reports in adolescents.[26]

Local immunomodulators present in cervicovaginal lavage fluid of those with STIs have been studied frequently in adults but not in adolescents. In this study, we observed a consistent (at enrollment and over time) relationship between the presence of any STI and increased local concentrations of IL-6, IL-1α, TGF-β, and IL-8, and decreased concentrations of IL-12. We also showed that adolescents with a STI had elevated numbers of PMNs in the lavage fluid. That is consistent with the increased local concentrations of IL-8 that is chemotactic for PMNs [27] and associated with the presence of TV. TV has been found to phagocytose lactobacilli, vaginal epithelial cells, leukocytes, erythrocytes and even GC both *in vivo* and *in vitro* [28,29] lending to a dominating role in local immunity. Given the association of most STIs with inflammation [4,5], the relationship between an STI and proinflammatory cytokines was expected. This was indeed evident in our study. The increase in proinflammatory cytokines in the presence of an STI is interesting, but increases in TGF-β and decreases in IL-12 may counter the proinflammatory effect. Thus, there is an eclectic influence that may or may not be evidence of a substantial response. However, when the individual STIs were evaluated, GC and CT were associated with an increase in only one or more proinflammatory cytokines, whereas TV was associated with increases in both proinflammatory and anti-inflammatory cytokines. These results suggest some response to the presence of the pathogen, albeit inconsistent and limited. A study by Hedges et al. [2] that examined local cytokine responses in adult women with GC found no significant differences in proinflammatory cytokines compared to uninfected women. Two separate studies, one examining HIV^+ adult women and the other examining HIV^+ adolescent females, have shown increases in IL-10 and IL-12 concentrations in genital secretions in those with non-ulcerative STIs [3,30] which we did not observe in this population. There are development and hormonal differences between adolescents and adults as well as variability in different adolescent cohorts. But what are similar in all cases are the relatively few changes in local immunomodulators in those with STIs although increases in some proinflammatory cytokines and the presence

of PMNs are indicative of some level or responsiveness.

Despite the observed relationships, or lack thereof, several limitations to this study should be noted. The middle adolescent girls included in this cohort represent a convenience sample of girls attending an adolescent health clinic and results may not be generalized to all adolescent girls. Specifically, prevalence rates of STIs, hormonal contraceptive use, and possibly sexual activity are expected to be higher on average in this cohort, given the nature of recruitment (at a health clinic, being treated for a STI, requesting/receiving contraceptives, etc). Furthermore, data on sexual activity and douching were based on self-report, which may have introduced a degree of misclassification bias due to social desirability and recall biases and data analysis had to be conducted by physical age since age at first menarche was not available. Nevertheless, this study represents the first of its kind to examine the association of sexual behaviors and clinical parameters with local immunomodulators. Another limitation is that we only examined the lower genital tract for immunomodulators although the cervix was included. Recognizing that CT is manifested more in the upper genital tract, the overall influences in the lower tract may be minimal providing an explanation for the lack of clear cause and effect relationships, at least for those with CT. Nonetheless, given the vast amount of data (and multiple analyses) showing no clear relationship between the parameters of interest and local immunity, further multivariate analyses were not undertaken. However, we recognize that the high STD rates could potentially be explained by other (measured or unmeasured) covariates and this should be explored in future analyses.

Acknowledgments

This work was supported by Public Health Service grant U19 AI43924 from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health.

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