Published in final edited form as:

Sex Transm Dis. 2008 July ; 35(7): 656–661. doi:10.1097/OLQ.0b013e31816b6593.

Immunogenetic Correlates of *Neisseria gonorrhoeae* Infection in Adolescents

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Abstract

Background—Understanding host factors modulating immunity to *Neisseria gonorrhoeae* infection may benefit work on vaccine development.

Methods—We analyzed longitudinal data collected from 485 male and female adolescents to determine genetic correlates of genital gonorrhea. Cytokine data from 388 females were analyzed to assess immunologic markers of gonorrhea and their relationship to genetic correlates.

Results—The T-G haplotype defining interleukin-2 (IL-2) gene promoter and intron 1 polymorphisms (−330T and −166G) was more frequently found in individuals who had gonorrhea (relative odds = 3.2, $P = 0.01$). Among 3 endocervical cytokines measured, IL-10 and IL-12 concentrations were higher and IL-2 lower when gonorrhea was detected. The decrease in endocervical IL-2 after gonorrhea acquisition was mostly restricted to subjects with the IL2 T-G haplotype, which may reflect involvement of a pathogen-specific and genetically mediated mechanism for differential IL-2 responses at genital mucosa. In addition, 2 human leukocyte antigen variants (Cw*04 and DQB1*05) were also independently associated with gonorrhea (adjusted relative odds = 1.9 and 0.5, respectively; $P < 0.05$ for both).

Conclusions—Confirmation of immunogenetic correlates of gonorrhea in larger cohorts may be useful in guiding further research on both innate and adaptive immune responses to *N. gonorrhoeae*.

Gonorrhea remains highly prevalent worldwide. In the United States, gonorrhea is the second most commonly reported bacterial sexually transmitted infection, with more than 330,000 cases reported in 2004, and nearly as many new infections not reported annually.^{1,2} Antibiotics recommended by the Centers for Disease Control and Prevention³ usually eradicate gonorrhea, but reinfection is common, suggesting that gonococcal infection in humans may fail to elicit long-lasting protective immunity, at least in some patients with modest levels of serum and genital mucosal anti-gonococcal antibodies after infection.^{4–10}

Studies on cellular immune responses in gonorrhea are sparse. In vitro and experimental studies of gonorrhea in humans (i.e., intraurethral challenge with *Neisseria gonorrhoeae*) have

generally demonstrated that proinflammatory cytokines predominate during acute infection.^{11–13} It remains unclear if such responses are applicable to genital mucosa in patients with sexually acquired gonorrhea.^{14,15}

Genetic factors, including human leukocyte antigen (HLA) alleles and/or cytokine gene polymorphisms, may account for variable immune responses to gonorrhea. HLA alleles contribute to the specificity of T-cell responses to gonococcal antigens, whereas cytokine gene polymorphisms can regulate cytokine production following immune activation. Among the sparse studies examining the relationship of genetic determinants to gonorrhea, Ness et al.¹⁶ evaluated data from the PEACH study of US women with clinically evident pelvic inflammatory disease. In a subset of patients, they found a borderline positive association of DQA1*0,301 and negative association of DQA1*0,102 with gonococcal cervicitis.¹⁶ Conceivably, these HLA class II alleles may differ in their ability to induce immune responses, especially humoral, to gonorrhea.

We previously investigated immunogenetic correlates of chlamydia in a cohort of adolescents enrolled in the Reaching for Excellence in Adolescent Care and Health (REACH) study.¹⁷ We subsequently assessed immunogenetic correlates of gonorrhea from this cohort and summarize our findings here.

Materials and Methods

Study Population and Data Collection

The REACH cohort comprised HIV-infected and high-risk HIV-uninfected male and female adolescents (13–18 years old) who were enrolled at 15 locations in 13 US cities.^{17,19–21} Longitudinal data were collected quarterly from 1996 to 2000, and patients with sufficient (6–56 months) follow-up, specimens for genetic analyses, and other relevant data were analyzed. Cytokine data were only available for female subjects.

N. gonorrhoeae Detection and Treatment

At enrollment and every 6 months thereafter, subjects were screened for *N. gonorrhoeae* (and *Chlamydia trachomatis*) by ligase chain reaction (LCx STD system, Abbott Laboratories, Abbott Park, IL).^{17,19} Test results from a central laboratory were returned to clinicians at variable time points (up to a 6-month interval). Treatment could be initiated before centrally obtained results became available because local testing was performed. Infected subjects were prescribed appropriate therapy.

DNA Extraction and HLA Typing

DNA extraction and HLA genotyping methods have been reported.^{17,22,23} Briefly, DNA was extracted from peripheral blood mononuclear cells for polymerase chain reaction (PCR)-based HLA typing: HLA-A, -B, and -C variants to 2-digit (group) specificity and DRB1 and DQB1 to 4-digit (allele) specificity.^{17,22,23} Haplotypes were assigned manually based on well-documented patterns of linkage disequilibria seen in various North American populations.^{24–27}

Classification of T_{H1} and T_{H2} Cytokine Gene Polymorphisms

Sixteen common single nucleotide polymorphisms (Supplemental Table) from 7 genes (IL2, IL4, IL4R, IL6, IL10, IL12B, and TNF) representing T_{H1} and T_{H2} pathways were typed by PCR with sequence-specific primers.²¹ Validation of techniques and individual genotyping followed procedures recommended by the manufacturer (Department of Transplantation Immunology, University of Heidelberg, Heidelberg, Germany and Pel-Freez Clinical Systems, LLC, Brown Deer, WI) and the 13th (2002) International Histocompatibility Workshop.²¹

^{28,29} When multiple single nucleotide polymorphisms within a single gene were tested, cis and trans relationships were established by PCR for all except 2 at the IL6 locus. The insertion or deletion variants in IL12B promoter sequence corresponded to 2 alleles (longer, L and shorter, S) defined by fragment sizes that differ by 4-bp after PCR amplification and automated denaturing gel electrophoresis on the AL-Fexpress system (Amersham Pharmacia Biotech, Piscataway, NJ).^{29,30}

Endocervical Cytokine Quantification

Endocervical secretions were collected every 6 months in a Weck-cel sponge placed in the cervix following speculum insertion as reported,³¹ and sponges were stored at -70°C . Before extracting secretions, sponges were weighed and equilibrated in phosphate buffered saline (PBS) + 0.25 mol/L NaCl with 10% Aprotinin (Sigma) for 30 minutes at 4°C . Secretions were extracted from each sponge by centrifuging at 12,000g for 20 minutes using a spin-x centrifuge filter unit. A previously described dilution factor for the final extract³² was used to calculate the final cytokine concentration when quantitative enzyme-linked immunosorbent assay kits (BioSource International, Camarillo, CA) were used to measure IL-2, IL-10, and IL-12 concentrations³³; the lower detection limits were 5, 0.2, and 0.8 pg/mL, respectively.³⁴ Other cytokines were not measured in REACH. We previously reported endocervical IL-2, IL-10, and IL-12 levels in chlamydia.^{18,35} In the analyses reported here, we focused on the relationship of these cytokines to gonorrhea, comparing cytokine levels at visits with gonorrhea and chlamydia coinfection, with either infection alone, or with neither present.

Statistical Analyses

We used SAS (Statistical Analysis Software, Version 8.5; SAS Institute, Cary, NC) for analyses. Individuals were classified as having had gonorrhea if they tested positive by gonococcal ligase chain reaction at least once. The relationships of gonorrhea to nongenetic (sociodemographic and clinical) factors (collected at baseline) and genetic markers were assessed univariately (χ^2 or Fisher exact tests) and with forward stepwise multivariable logistic regression.

Analyses of gonorrhea and other infection categories with endocervical cytokine responses were initially performed by selecting a single visit for each subject whose infection could be categorized as defined above (gonorrhea and chlamydia coinfection, gonorrhea or chlamydia alone, or neither). In subjects with visits in more than 1 of these categories, assignment was made to a single category in the priority order listed above based on the category frequency (i.e., preference was given to coinfection as the least frequent category, then gonorrhea alone, etc.). For subjects with more than 1 visit in a chosen category (e.g., multiple visits with gonorrhea alone), a single visit was selected for study randomly (by assigning a random number to each visit and selecting the visit with the lowest random number); we performed additional analyses to verify the randomization by choosing a different visit from the multiple same infection category visits and found it to be effective with no difference in study findings (these verification analyses are not shown). Because cytokine levels did not follow a normal distribution,³⁶ median cytokine levels were assessed by the Kruskal-Wallis test. We subsequently tested $\log_{10}$ -transformed IL-2, IL-10, and IL-12 levels in multivariable repeated measures (multiple-visit) regression models, in which distinctive infection categories and genetic and nongenetic factors were evaluated for independent effects.

Results

Characteristics of Study Participants and Relationship to *N. gonorrhoeae* Infection

Clinical and genetic data were completed for 485 adolescents with characteristics previously reported.¹⁷ Subjects were predominantly female (74%), black (70%), and HIV-positive (68%),

with a mean age of 17 years and median follow-up of 35 (range 6–56) months. An average of 6 (± 2) *N. gonorrhoeae* and *C. trachomatis* tests were performed, and were positive at least once in 104 (21%) and 199 (41%) of subjects, respectively.

Subjects with versus without gonorrhea differed in relation to sociodemographic and clinical characteristics (Table 1). Those with gonorrhea were more likely to be black ($P = 0.015$), ≥ 17 years or older ($P = 0.0002$), HIV-positive ($P = 0.001$) with CD4+ T-cell counts $\geq 400/\mu\text{L}$, and on highly active antiretroviral therapy; they also more frequently acquired chlamydia ($P < 0.0001$) and had more gonococcal tests performed and longer follow-up ($P = 0.002$ and $P = 0.0008$, respectively) (duration of follow-up was retained as a covariate in subsequent analyses as it could be universally applied to all subjects).

Genetic Variants Associated with *N. gonorrhoeae* Infection—Univariate Analyses

The –1902G variant in interleukin-4 receptor gene (*IL4R*) and the interleukin-2 gene (*IL2*) haplotype defined by –330T and 166G (*IL2* T-G) were present more often in those with gonorrhea, whereas the –330G and –166T variants in *IL2* as well as the *IL2* T-T and G-G haplotypes were present less often (all $P < 0.05$) (Table 2). Other cytokine gene variants or haplotypes present in $\geq 5\%$ of patients were not associated with gonorrhea status. In addition, 4 HLA alleles (A*23, B*53, Cw*04, and DRB1*15) and 3 haplotypes (B*53-Cw*04, B*44-Cw*04, and DRB1*15-DQB1*06) were present more often in subjects with gonorrhea (Table 2), whereas 2 alleles (A*02 and DQB1*05) were present less often (all $P < 0.05$) (Table 2). DQB1*06 showed a borderline association with gonorrhea ($P = 0.052$). The stronger association of gonorrhea with DRB1*15 versus DQB1*06 suggested that the DRB1*15-DQB1*06 haplotypic effect was mostly driven by DRB1*15, although strong linkage disequilibrium precluded a clear separation of allelic effects. Other HLA determinants present in $\geq 5\%$ of the population were not associated with gonorrhea status.

Relationship of Genetic and Nongenetic Determinants to *N. gonorrhoeae* Infection as Defined in Multivariable Models

Individual genetic and nongenetic factors associated with gonorrhea in univariate analyses were tested in a multivariable logistic regression model (Table 3). Neither race nor several genetic determinants remained significantly associated with gonorrhea when examined jointly. In the final (reduced) model, gonorrhea was significantly more likely in subjects age ≥ 17 ($P = 0.0002$), HIV-1 infected ($P = 0.011$), with longer follow-up ($P = 0.007$), with chlamydia during the study ($P < 0.0001$), with the *IL2* T-G haplotype ($P = 0.012$), and with Cw*04 ($P = 0.008$). Subjects with DQB1*05 were significantly less likely to have gonorrhea ($P = 0.022$).

Relationship of Endocervical Cytokine Concentrations to *N. gonorrhoeae* Infection and Comparison With Those with *C. trachomatis* Infection, *N. gonorrhoeae* and *C. trachomatis* Coinfection, and No infection

In analyses of females with complete cytokine data ($n = 388$; the analyses also included some females not included in the above genetic analyses because of incomplete genetic data), we evaluated endocervical IL-2, IL-10, and IL-12 concentrations at a single visit for each female in 1 of the 4 infection categories: gonorrhea and chlamydia coinfection, gonorrhea or chlamydia alone, or neither (see the Materials and Methods section). For each cytokine, the median concentration differed significantly for each different infection category (Table 4). IL-2 levels were highest in those without chlamydia or gonorrhea and lowest in those with coinfection. IL-10 levels were lowest in those with neither infection and highest in those with coinfection. IL-12 levels were lowest in those with neither infection but similar in those with single- and coinfection. A subanalysis of 48 females with gonorrhea alone who then cleared infection within 2 consecutive visits (a visit with gonorrhea followed by a subsequent visit without gonorrhea detected) revealed that median IL-2 levels increased (217.5 vs. 234.6 pg/mL) while

median IL-10 and IL-12 levels decreased (95.9 vs. 43.5 pg/mL and 340.7 vs. 278.5 pg/mL, respectively) after gonorrhea clearance; only the IL-10 decrease reached statistical significance ($P = 0.005$).

We performed multivariable analyses on all females using repeated measures with $\log_{10}$ -transformed endocervical IL-2, IL-10, and IL-12 concentrations as dependent variables and independent covariates that included the 4 infection categories, relevant genetic determinants, and other nongenetic factors that could influence cytokine levels [including other STIs, demographic and behavioral factors, oral contraceptive use, and immune status (HIV infection and CD4+ T-cell count)]. After controlling for covariates (Table 5): 1) IL-2 concentrations were significantly lower ($P = 0.004$) in those with chlamydia alone ($P = 0.002$) or coinfection ($P = 0.004$) compared with no chlamydia or gonorrhea; IL-2 levels were also somewhat lower in those with gonorrhea alone compared with neither infection ($P = 0.09$); 2) IL-10 levels were significantly higher in those with gonorrhea and/or chlamydia than in those with neither (all $P \leq 0.01$); 3) IL-12 levels were significantly higher in those with gonorrhea and chlamydia coinfection versus neither infection ($P = 0.01$); 4) HLA Cw*04 and DQB1*05 were not significantly associated with cytokine levels, except for a trend toward higher IL-2 concentrations in those with DQB1*05 ($P = 0.055$); and 5) the *IL2* T-G haplotype was significantly associated with lower IL-2 levels ($P = 0.012$). The magnitude of the cytokine responses was similar in gonorrhea alone versus chlamydia alone, with differences in P values mainly because of sample size.

Further, in a subset of subjects ($n = 43$) who acquired gonorrhea within 2 consecutive visits (a visit with no gonorrhea followed by a subsequent visit with gonorrhea detected), those with the *IL2* T-G haplotype ($n = 39$) had lower median IL-2 concentrations after gonorrhea acquisition (246.9 vs. 293.6 pg/mL), whereas those without *IL2* T-G ($n = 4$) had higher median IL-2 concentrations after gonorrhea acquisition (161.6 vs. 132.7 pg/mL); these differences were not statistically significant ($P = 0.29$ for analyses of $\log_{10}$ IL-2 concentrations).

Discussion

Our analyses identified associations of several genetic variants with gonorrhea in males and females and patterns of mucosal cytokine responses to gonorrhea in females. A single interleukin-2 gene (*IL2*) haplotype (T-G) and 2 HLA variants, Cw*04 and DQB1*05, were independently associated with gonorrhea. A possible link between mucosal IL-2 levels in females and a haplotype in the gene encoding IL-2 is of particular interest. Unfortunately, cytokine data were not available for males.

Comparison of endocervical cytokine levels in females with and without gonorrhea produced clear evidence that IL-2, a T-cell growth factor and a pro- T_H1 cytokine, may be a limiting factor in immune responses to *N. gonorrhoeae*. The *IL2* T-G haplotype, associated with a threefold higher risk for gonorrhea in our study, has previously been associated with reduced production of IL-2,³⁷ and the latter relationship was also demonstrated in our repeated measures analyses (Table 5). In contrast, a slightly increased IL-2 response was seen in females with DQB1*05, which was associated with a twofold lower risk for gonorrhea. Such genetically mediated differences in mucosal IL-2 responses following *N. gonorrhoeae* infection are consistent with the important role IL-2 can play in regulating antigen-specific T-cells, B-cells, and NK cells.^{38,39} Overall, it seemed that a blunted IL-2 response related to the *IL2* T-G haplotype or enhanced IL-2 response partially attributable to DQB1*05 may impair or boost expression of T_H1 cytokines and the ability of NK cells to effectively prevent *N. gonorrhoeae* infection. It is also possible that other nongenetic factors, including other cytokines, may influence IL-2 levels.

Endocervical IL-10 and IL-12 concentrations were not associated with specific cytokine or HLA genotypes in our study. Of note, in females coinfecting with chlamydia and gonorrhea, IL-2 and IL-10 levels showed additive effects, with a greater decrease and increase in respective levels of IL-2 and IL-10 when compared with others with either infection alone. These mucosal pathogens, which both infect genital tract columnar epithelial cells, seem to elicit similar immune responses in females, despite clear differences in genetic correlates. Thus, different mechanisms can drive and induce similar immunologic phenotypes.

The only previously published study (the PEACH cohort) that evaluated influences of HLA on gonorrhea¹⁶ differed from ours in that it targeted adolescent and adult females with clinical evidence of pelvic inflammatory disease, in contrast to the HIV-infected and high-risk HIV-uninfected adolescents with uncomplicated gonorrhea in our study. From the PEACH cohort, DQA1*0,102 and DQA1*0,301 were associated with gonococcal cervicitis¹⁶; these *DQA1* alleles are known to be in strong linkage disequilibrium with DQB1*05/*06 and DQB1*03, respectively.²⁴ As DQB1*05 was implicated in our study, the haplotype including DQA1*0,102 and DQB1*05 may be a favorable factor in both adolescent and adult populations. Further, the association of HLA-Cw*04 with increased risk of gonorrhea we identified has not been reported elsewhere. This novel relationship is worth further investigation as Cw*04 is a common variant in all major human populations.

Certain genetic relationships seen here were less likely than others to have been observed by chance in view of previous studies by others (e.g., *DQA1* in gonorrhea¹⁶) and by us (e.g., IL-2 in chlamydia³⁵). Clearly, those relationships that appear consistent with reported evidence deserve greater attention than those without (e.g., Cw*04). With overrepresentation of HIV-positive patients in our population, the immunogenetic factors identified in this study could be more relevant to studies involving immunosuppressed patients. It will be important to repeat such studies in HIV-negative immunocompetent patient cohorts.

Acknowledgments

The authors thank investigators and staff [listed in *J Adolesc Health* 2001. 29 (suppl): 5 to 6] of the Adolescent Medicine HIV/AIDS Research Network (1994–2001) and the youth who participated in the REACH project for their valuable contributions. They are further indebted to E. Lobashevsky, A.D. Myracle, and C.A. Rivers for excellent technical assistance.

Supported by grant U01 HD32830 from the National Institute of Child Health and Human Development, with additional funding from the National Institute on Drug Abuse, National Institute of Allergy and Infectious Diseases, and National Institute of Mental Health.

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TABLE 1

Characteristics of the Study Population Consisting of 485 Adolescents, Stratified by Occurrence of *Neisseria gonorrhoeae* Infection Between 1996 and 2000

Patient Characteristic	<i>Neisseria gonorrhoeae</i> Infection		<i>P</i>
	Negative	Positive	
Age at baseline			
<17 yr	131 (34.4)	16 (15.4)	0.0002
≥17 yr	250 (65.6)	88 (84.6)	Referent
Race			
African-American	257 (67.5)	83 (79.8)	0.015
Other	124 (32.6)	21 (20.2)	Referent
Gender			
Male	96 (25.2)	28 (26.9)	0.70
Female	285 (74.8)	76 (73.1)	Referent
Sex partners [*]			
<2	270 (72.0)	75 (73.5)	0.80
≥2	105 (28.0)	27 (26.5)	Referent
History of gonorrhea			
No	311 (81.6)	79 (76.0)	0.20
Yes	70 (18.4)	25 (24.0)	Referent
Genital chlamydia			
No	249 (65.4)	37 (35.6)	<0.0001
Yes	132 (34.7)	67 (64.4)	Referent
Months of follow-up (mean ± SD)	30.0 ± 14.5	35.0 ± 14.4	0.0008
Number of gonorrhea tests (mean SD)	5.1 ± 2.4	5.9 ± 2.3	0.002
HIV-1 infection			
Seropositive	245 (64.3)	84 (80.8)	0.001
Seronegative	136 (35.7)	20 (19.2)	Referent
HAART ^{*†}			
No	187 (76.3)	45 (54.2)	0.0001
Yes	58 (23.7)	38 (45.8)	Referent
CD4 ⁺ T-cells			
≥400/μL	93 (24.4)	41 (39.4)	0.002
<400/μL	288 (75.6)	63 (60.6)	Referent
OCP use in past 3 mo			
No	166 (58.9)	46 (61.3)	0.70
Yes	116 (41.1)	29 (38.7)	Referent

Note: Data are presented as number (%).

* Information is missing for some subjects.

[†]HAART is only relevant to HIV-1 seropositive individuals. SD indicates standard deviation; HIV-1, human immunodeficiency virus type 1; HAART, highly active antiretroviral therapy; OCP, oral contraceptive pill.

TABLE 2Immunogenetic Correlates of *Neisseria gonorrhoeae* Infection in 485 Adolescents Based on Univariate Analyses

Locus	Variant	<i>Neisseria gonorrhoeae</i> Infection		<i>P</i>
		Negative	Positive	
HLA-A	*02	150 (39.4)	30 (28.9)	0.049
HLA-A	*23	62 (16.3)	27 (26.0)	0.024
HLA-B	*53	74 (19.4)	32 (30.8)	0.047
HLA-Cw	*04	132 (34.8)	54 (51.9)	0.001
HLA-DRB1	*15	78 (20.5)	33 (31.7)	0.015
HLA-DQB1	*05	124 (32.6)	20 (19.2)	0.008
HLA-DQB1	*06	143 (37.5)	50 (48.1)	0.052
<i>IL2</i>	−330G	81 (21.4)	11 (10.8)	0.015
<i>IL2</i>	−166T	99 (26.2)	16 (15.7)	0.027
<i>IL4R</i>	−1902G	288 (75.8)	90 (86.5)	0.019
HLA-B-Cw	*44-∗04	20 (5.3)	12 (11.5)	0.022
HLA-B-Cw	*53-∗04	61 (16.0)	29 (27.9)	0.006
HLA-DRB1-DQB1	*15-∗06	77 (20.2)	33 (31.7)	0.013
<i>IL2</i>	−330T−166T	99 (26.2)	16 (15.7)	0.027
<i>IL2</i>	−330T−166G	315 (83.3)	96 (94.1)	0.006
<i>IL2</i>	−330G−166G	81 (21.4)	11 (10.8)	0.015

Note: Data are presented as number (%).

TABLE 3Correlates of *Neisseria gonorrhoeae* Infection Among 485 Adolescents Based on Multivariable Analyses

Host Factors Tested in Model *	Full Model		Reduced Model	
	RO (95% CI)	P	RO (95% CI)	P
Race (African-American vs. other)	1.43 (0.76, 2.70)	0.3	N/A [†]	—
Age (<17 vs. ≥17)	3.65 (1.88, 7.09)	0.0001	3.42 (1.80, 6.50)	0.0002
HIV-1 infection (positive vs. negative)	2.00 (1.08, 3.68)	0.027	2.14 (1.19, 3.85)	0.011
Follow-up after enrollment (per year)	1.36 (1.10, 1.69)	0.005	1.33 (1.08, 1.65)	0.007
Genital chlamydia (yes vs. no)	2.97 (1.79, 4.93)	<0.0001	3.31 (2.02, 5.43)	<0.0001
HLA-A*02 (n = 180)	0.73 (0.42, 1.27)	0.30	N/A [†]	—
HLA-A*23 (n = 89)	1.38 (0.75, 2.55)	0.30	N/A [†]	—
HLA-B*53 (n = 106)	1.04 (0.54, 2.03)	0.90	N/A [†]	—
HLA-Cw*04 (n = 186)	1.80 (1.01, 3.21)	0.045	1.93 (1.18, 3.14)	0.008
HLA-DQB1*05 (n = 144)	0.48 (0.26, 0.88)	0.017	0.51 (0.29, 0.91)	0.022
IL4R-1902G variant (n = 378)	1.64 (0.82, 3.28)	0.16	N/A [†]	—
HLA-DRB1*15-DQB1*06 (n = 110)	1.57 (0.89, 2.75)	0.12	N/A [†]	—
IL2-330T-166G haplotype (n = 411)	2.65 (1.00, 7.00)	0.050	3.21 (1.29, 8.00)	0.012

* In univariate models, these factors showed statistically significant ($P \leq 0.05$) associations with *Neisseria gonorrhoeae* infection (see Table 1 and Table 2).

[†] Dropped out of model when adjusted $P > 0.05$.

HIV-1 indicates human immunodeficiency virus type 1; RO, relative odds; CI, confidence interval; N/A, not applicable.

TABLE 4

Endocervical IL-2, IL-10, and IL-12 Concentrations in 388 Female Adolescents in the Presence of Gonorrhea and/or Chlamydia Infection

Infection Status	Cytokine *	N	Median (25th, 75th percentile) [†]	P [‡]
CT and GC coinfection	IL-12	27	300.2 (165.7, 485.5)	0.003
No CT or GC	IL-2	227	282.5 (152.7, 507.2)	
CT alone	IL-2	89	224.1 (95.0, 385.5)	
GC alone	IL-2	40	272.3 (115.2, 390.0)	
CT and GC coinfection	IL-2	27	153.6 (56.0, 355.4)	<0.0001
No CT or GC	IL-10	229	30.4 (0.10, 97.6)	
CT alone	IL-10	90	66.0 (17.1, 144.7)	
GC alone	IL-10	42	84.1 (29.6, 245.1)	
CT and GC coinfection	IL-10	27	98.3 (16.3, 204.7)	<0.0001
No CT or GC	IL-12	229	140.0 (37.3, 319.2)	
CT alone	IL-12	90	290.7 (120.2, 491.5)	
GC alone	IL-12	42	304.0 (127.0, 544.6)	

* Five subjects had insufficient sample available for IL-2 testing at the specified visit.

[†] Concentrations are expressed as pg/mL.

[‡] Based on the Kruskal-Wallis test.

CT indicates chlamydia; GC, gonorrhea.

Multivariable Repeated Measures (Multiple-Visit) Regression Analysis Assessing the Influence of Genetic Factors and Gonorrhea and/or Chlamydia on Repeated Measures of Endocervical IL-2, IL-10, and IL-12 Concentrations*

Covariates [†]	IL-2 (n = 1660 [‡])		IL-10 (n = 1680 [‡])		IL-12 (n = 1680 [‡])	
	B [§]	P	B [§]	P	B [§]	P
HLA-Cw*04 (yes vs. no)	-0.023	—	0.074	—	0.017	—
HLA-DQB1*05 (yes vs. no)	0.058	0.055	-0.089	—	-0.002	—
IL2 (-330)T + 166G haplotype (yes vs. no)	-0.107	0.012	N/A	N/A	N/A	N/A
Gonorrhea and chlamydia coinfection	-0.335	0.004	0.724	0.007	0.534	0.010
Chlamydia infection alone	-0.15	0.002	0.450	0.0001	0.063	—
Gonorrhea infection alone	-0.157	0.093	0.598	0.006	-0.102	0.195

* Cytokine concentrations are log10 transformed in the analyses.
[†] Analyses also controlled for age, race, number of sexual partners, HIV infection status, CD4⁺ cell count, oral contraceptive pill use, cervical HPV infection, bacterial vaginosis, and trichomoniasis.
[‡] Number of measures available.
[§] Slopes of cytokines in response to *Neisseria gonorrhoeae* infection.
^{||} P ≥ 0.20 are omitted (—).
N/A indicates not applicable.