MAJOR ARTICLE

Immunoepidemiologic Profile of *Chlamydia trachomatis* Infection: Importance of Heat-Shock Protein 60 and Interferon-γ

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Epidemiological, animal, and in vitro investigations suggest that *Chlamydia trachomatis* infection engenders acquired immunity, the basis for which is incompletely defined, especially in humans. In a prospective cohort study of women at high risk for *C. trachomatis* infection, we found that, at baseline and after adjustment for age and other potential confounding variables, production of interferon-γ by peripheral-blood mononuclear cells (PBMCs) stimulated with chlamydia heat-shock protein 60 strongly correlated with protection against incident *C. trachomatis* infection. This investigation supports a direct role for *C. trachomatis*–specific immune responses in altering the risk of infection and suggests immune correlates of protection that are potentially useful in vaccine development.

Chlamydia trachomatis is the most common bacterial sexually transmitted infection (STI), with an estimated 92 million cases occurring globally each year, including 3 million in the United States [1, 2]. Prevention of *C. trachomatis* infection remains a top public-health priority, because infections may be recurrent or persistent, cause adverse reproductive consequences, enhance the transmission of HIV, and contribute to human papillomavirus–induced cervical neoplasia [3–7]. Vaccine development is critical for chlamydia control but is dependent, in part, on a sound understanding of the immune correlates of protection. Numerous studies in murine models have demonstrated the particularly important role played by T cell–mediated immune responses in host defense against infection [8–13]. Human strains of *C. trachomatis* are sensitive to growth inhibition in vitro by interferon (IFN)–γ, suggesting that Th1 cytokines such as IFN-γ are likely important in protection [14]. The immunological paradigm for chlamydia immunity that is emerging from murine studies involves the roles played by (1) $CD4^+$ Th1 effector cells that secrete IFN-γ, tumor necrosis factor–α and –β, and interleukin (IL)–2 in the clearance of infection and (2) $CD4^+$ Th1 cells, together with antigen-specific B cells, in resistance to reinfection [8–21].

Limited immunoepidemiological data have supported the relevance of these murine data to the immunobiological characteristics of human chlamydia infection [3, 22–26]. In particular, studies have shown that individuals with severe trachomatous scarring exhibit higher antigen-specific Th2 IL-4 production and impaired lymphoproliferative responses, compared with control subjects [25], and that HIV-infected individuals with $CD4^+$ T cell depletion exhibit an increased risk of

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clinical pelvic inflammatory disease (PID) when coinfected with *C. trachomatis* [3]. Overall, human data from cross-sectional investigations support the hypothesis that it is the characteristics of the immunological response, such as Th1 versus Th2 polarization, to chlamydia antigens that determine susceptibility and resistance to *C. trachomatis* infection; nevertheless, no prospective cohort investigation has been conducted to test this hypothesis. Therefore, we enrolled a cohort of highly exposed commercial sex workers, to test for correlations between chlamydia-specific cellular and humoral immune responses and protection against infection.

PARTICIPANTS, MATERIALS, AND METHODS

Study population. The study protocol was approved by the institutional review boards for human subjects at the University of Washington, the University of Manitoba, and the University of British Columbia and by the Ethical Review Committee at Kenyatta National Hospital, Nairobi, Kenya. Procedures followed were in accordance with the ethical standards for human experimentation established by the Declaration of Helsinki of 1975 (revised in 1983). A cohort of 299 commercial sex workers, 18–35 years old, was formed beginning in May 2000 at the Kariobangi Nairobi City Council Clinic, Nairobi, Kenya, to study the epidemiological and immunobiological profiles of STIs. Women were counselled on the hazards of commercial sex work and were encouraged to seek alternate employment. They were also counselled on how to reduce harm, were provided free condoms, and were given treatment for bacterial STIs. After written, informed consent was obtained, the women made an initial visit, which included collection of demographic and clinical data and a general physical and pelvic examination. The women were asked to return for evaluation of incident STI every 2 months. At the initial visit, cervical specimens were obtained for *C. trachomatis* and *Neisseria gonorrhoeae* molecular detection; endocervical mucus was obtained by use of a pipette (Aspirette Unimar) for measurement of levels of antibodies to chlamydia elementary body (EB) and chlamydia heat-shock protein 60 (CHSP60), and serum was collected for HIV-1 serological testing, $CD4^+$ and $CD8^+$ T cell enumeration, *C. trachomatis* antibody (to EB and CHSP60) testing, and to obtain peripheral-blood mononuclear cells (PBMCs) for antigen-specific cytokine production assays. At each follow-up visit, interval clinical and sexual histories and symptoms related to STIs were ascertained. The women were examined for evidence of an STI, which included molecular testing for *C. trachomatis* and *N. gonorrhoeae.* At 6-month intervals, serum collection for HIV-1 and syphilis serological testing and for $CD4^+$ T cell enumeration was repeated. The women were asked to return to the study clinic within 4 days to receive the results of STI testing. Those found to be infected with *C. trachomatis* or *N. gonorrhoeae* received 100 mg of doxycycline twice daily for 7 days or a single 500-mg dose of ciprofloxacin, respectively.

C. trachomatis and *N. gonorrhoeae* DNA was detected by a commercially available polymerase chain reaction (PCR) assay (Amplicor; Roche Diagnostic Systems). Serum was tested for antibodies to HIV-1 by ELISA (Detect HIV-1; Biochem ImmunoSystems). Those found to be positive by the initial screening test had a second, confirmatory test performed (Recombigen; Cambridge Biotech). For serological testing for syphilis, the Rapid Plasma Reagin assay (Becton Dickinson) was used for initial screening, and a *Treponema pallidum* hemagglutination assay (Biotech Laboratories) was used for confirmation.

Antibodies to EB and CHSP60. Endocervical mucus at a 1:10 dilution and serum at a 1:200 dilution were tested for specific antibodies to EB and CHSP60 by a modified ELISA, as described elsewhere [22].

Purification of PBMCs and analysis of T cell subsets. PBMCs were purified from serum by use of ficoll-hypaque. Absolute T cell subsets ($CD4^+$ and $CD8^+$ T cells) were enumerated by FACScan analysis (Becton Dickinson), in accordance with standard procedures.

Antigen preparations. EB was prepared with laboratory strains that belonged to serovars E, F, K, and L2 EB, as described elsewhere [22]. The CHSP60 gene was cloned from serovar D genomic DNA into pET Vector (NOVagen). The histidine-tagged recombinant protein was expressed in *Escherichia coli* BL21 and was purified by use of a nickel–nitrilotriacetic acid agarose column (QIAGEN).

Antigen-specific cytokine production by PBMCs. To establish the optimal stimulating concentrations of EB and CHSP60 for the cytokine response assay, PBMCs were challenged with concentrations of EB ranging from 4×10^5 to 4×10^3 IFN units and concentrations of CHSP60 ranging from 10 to 0.1 μg/mL. The minimal concentrations that stimulated the highest production of IFN-γ were chosen as the optimal stimulating concentrations of EB and CHSP60. PBMCs were cultured in round-bottom 96-well plates (Corning) at a concentration of 5×10^5 cells/mL in RPMI 1640 supplemented with 10% fetal calf serum, 50 mmol/L 2-mercaptoethanol, 100 μg/mL penicillin, and 12 μg/mL gentamicin in both the presence and absence of various antigens: EB from serovars E, F, K, and L2 (equally mixed), in a total protein concentration of 1 or 0.5 μg/mL CHSP60. After 5 days of culture, supernatants from PBMC culture wells were harvested and stored at -80°C for later use in IFN-γ, IL-5, IL-10, and IL-13 ELISAs. Paired antibodies for human IFN-γ, IL-5, IL-10, and IL-13 ELISAs were purchased from PharMingen. An assay result >2 times that of the negative control was considered to be positive. Cytokines were measured in picograms per milliliter.

Data analysis. For data analysis, we used SPSS for Windows (version 11.5; SPSS) and S-Plus for Windows (version

Table 1. Sociodemographic characteristics, sexual history, and laboratory findings at baseline in a cohort of commercial sex workers in Nairobi, Kenya.

Characteristic	Value[a]
Age, mean ± SD, years	23.9 ± 5.3
Years lived in Nairobi, mean ± SD	14.1 ± 9.1
Years of prostitution, mean ± SD	3.9 ± 3.2
Clients per week, mean ± SD, no.	11.7 ± 7.6
Charge per sex act, mean ± SD, Kenyan shillings	179 ± 147
Age at menarche, mean ± SD, years	14.6 ± 1.7
Age at first intercourse, mean ± SD, years	15.6 ± 1.9
Marital status	
Single (never married)	204 (68)
Married	1 (0.3)
Widowed/divorced/separated	93 (31)
Place of work	
Bar	235 (79)
Nightclub	27 (9)
Home	21 (7)
Other	15 (5)
Percentage of condom usage with clients per week	
Never	4 (1)
1%–24%	22 (8)
25%–49%	55 (18)
50%–74%	94 (32)
75%–99%	57 (19)
Always	65 (22)
Have a regular partner	162 (54)
Regular partners per week, mean ± SD, no.	1.4 ± 1.1
Percentage of condom usage with regular partners	
Never	99 (33)
1%–24%	16 (5)
25%–49%	4 (1)
50%–74%	11 (4)
75%–99%	3 (1)
Always	22 (7)
Pregnant at enrollment	2 (1)
Current family planning method	
None	90 (30)
Depo-Provera	93 (31)
Birth control pills	75 (25)
Condoms	37 (12)
Tubal ligation	5 (2)
Intrauterine contraceptive device	3 (1)
Other	3 (1)
Douche	253 (85)
Have sex during menses	70 (24)
Have anal sex	34/267 (11)
Use intravenous drugs	2 (1)
Smoke cigarettes	62 (24)
Drink alcohol	
Never	85 (28)
1–2 drinks/week	201 (67)
3–4 drinks/week	3 (1)
1–2 drinks/day	7 (2)
>2 drinks/day	3 (1)
Health problems during past year	136 (46)
Vaginal discharge	70 (23)
Painless genital ulcer	13 (4)
Severe abdominal pain	70 (23)
Tuberculosis	3 (2)
Syphilis	23/287 (8)
HIV-1 seropositive	87/288 (30)
Chlamydia trachomatis infection	24 (8)
Neisseria gonorrhoeae infection	18 (6)

NOTE. Data are no. (%) of participants, unless otherwise noted.

[a] The sample size for all data is 299, unless a different denominator is given.

6.0; Mathsoft). Several laboratory measures were log transformed, resulting in more-centralized values. Tests for associations with continuous measures included correlation coefficients and Mann-Whitney *U* tests. For categorical variables, χ^2 tests were performed. Graphical examination aided in determination of the form of each measure's association with *C. trachomatis* infection. Survival analysis of recurrent events (of the same type) was performed to assess factors related to *C. trachomatis* infection. Participants were considered to be at risk for new infections from the time of the previous infection (counting process formulation), and adjustments were made to account for the relatedness of multiple infections occurring in the same participants.

RESULTS

Cohort characteristics. Table 1 summarizes the sociodemographic characteristics, sexual history, and laboratory findings for the 299 women in the cohort at enrollment; 87 (30%) were infected with HIV-1, 23 (8%) had syphilis, and 24 (8%) and 18 (6%) were infected with *C. trachomatis* and *N. gonorrhoeae,* respectively.

Forty-three cases of incident *C. trachomatis* infection were detected during 307 women-years of observation (annual incidence, 14.0%). After 12 months and 24 months, respectively, 85% (95% confidence interval [CI], 80%–90%) and 80% (95% CI, 74%–85%) remained uninfected by *C. trachomatis;* thus, most infections occurred during the first year of observation (figure 1). Overall, 133 women (44%) attended all scheduled follow-up visits, 63 (21%) missed a single visit, 37 (12%) missed 2 visits, 34 (11%) missed 3 visits, and 32 (11%) missed ⩾4 visits. The first model used to calculate incidence assumed that women were negative for *C. trachomatis* at missed visits, an assumption that probably led to an estimate that was lower than the true rate of infection. For comparison, we repeated the calculations censoring participants after they had missed a

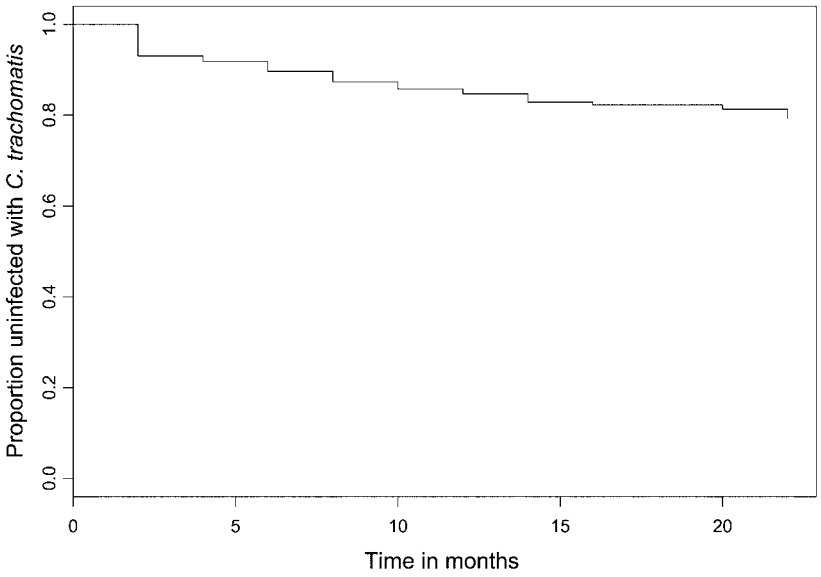

Figure 1. Survival table demonstrating time to first *Chlamydia trachomatis* infection

single follow-up visit. This method left 30 cases of *C. trachomatis* infection with 125 women-years of observation, yielding an annual incidence of 24.0%—an estimate that was 58% greater than that found by the initial model and that most likely was higher than the true rate of infection.

Risk factors for incident* C. trachomatis *infection. Younger age and a history of prostitution of ⩽2 years were significantly associated with an increased risk of incident *C. trachomatis* infection (table 2). Marital status, workplace location, number of clients per week, current family planning method, and reported condom use with clients (⩾75% vs. <75%) were not associated with the risk of incident *C. trachomatis* infection. *C. trachomatis* infection at enrollment (hazard ratio [HR], 9.1 [95% CI, 4.5–18.4]) and *N. gonorrhoeae* infection detected during follow-up (HR, 3.4 [95% CI, 1.1–10.4]) were associated with a significantly increased risk of incident *C. trachomatis* infection (table 2). HIV-1 infection itself did not correlate with the risk of incident *C. trachomatis* infection.

Table 2. Hazard ratios (HRs) and 95% confidence intervals (CIs) from a Cox proportional hazards model testing for demographic, sexual, clinical, and laboratory risk factors for incident *Chlamydia trachomatis* infection in a cohort of commercial sex workers in Nairobi, Kenya.

Risk factor	HR (95% CI)
Age	0.92 (0.85–1.00)
Years lived in Nairobi	0.98 (0.93–1.03)
Single (never married)	2.3 (0.8–6.7)
Place of work, nightclub	0.8 (0.5–1.2)
⩽2 years duration of prostitution	1.6 (1.0–2.4)
⩽4 clients per week	1.0 (0.6–1.6)
Charge ⩽150 Kenyan shillings per sex act	1.2 (0.8–1.8)
No. of regular partners	1.1 (0.5–2.6)
Condom use reported <75% with client	1.5 (1.0–2.3)
Not currently using birth control	0.8 (0.4–1.6)
Ever smoke cigarettes	1.4 (0.5–3.9)
Ever drink alcohol	1.0 (0.4–2.4)
Health problems last year	0.8 (0.4–1.5)
Incident case of syphilis	0.3 (0.04–2.6)
HIV-1 seropositive	1.5 (0.6–3.8)
C. trachomatis infection at enrollment	9.1 (4.5–18.4)
Incident *Neisseria gonorrhoeae* infection during follow-up	3.4 (1.1–10.4)

NOTE. CI, confidence interval.

For a subset of women (n = 130–173), baseline specimens were collected and studied for cytokine production by PBMCs after stimulation with antigen. IL-10 was the most prevalent cytokine detected, with 75% and 78% of specimens assayed found to be positive after stimulation with EB and CHSP60, respectively (table 3). For all of the measured cytokines except IL-10, the percentage of specimens with a positive response was greater after stimulation with EB than after stimulation with CHSP60; for example, IFN-γ was detected in 40% of specimens after stimulation with EB, compared with 18% after stimulation with CHSP60.

Associations with incident *C. trachomatis* infection were explored by a survival analysis comparing those with and without production of a given cytokine by stimulated PBMCs. For the survival analysis, participants were censored at the first missed visit. IFN-γ production (HR, 0.2 [95% CI, 0.03–1.0]) (figure 2*A*) and IL-10 production (HR, 6.7 [95% CI, 1.0–47.1]) (figure 2*B*) after stimulation with CHSP60 and IL-13 production (HR, 0.2 [95% CI, 0.1–0.7]) (figure 2*C*) after stimulation with EB were significantly associated with an altered risk of incident *C. trachomatis* infection (table 4). The IFN-γ response to CHSP60 and the IL-13 response to EB positively correlated with each other (r = 0.49; P < .001). In multivariate analysis, after adjustment for age, years lived in Nairobi, single (never married) marital status, and both cytokines, the IFN-γ response to CHSP60 (adjusted HR [AHR], 0.2 [95% CI, 0.02–1.0]) and the IL-13 response to EB (AHR, 0.2 [95% CI, 0.1–0.8]) were independently and significantly associated with a reduced risk of *C. trachomatis* infection; the association with IL-10 production failed to reach statistical significance (AHR, 5.3 [95% CI, 0.8–34.5]). We repeated the survival analysis assuming that participants were negative for *C. trachomatis* at missed visits; HRs and CIs did not differ greatly when this second method was used, excepting those for IL-10, which maintained a high HR but was not statistically significant (P = .27).

Levels of antibodies to EB and CHSP60 in endocervical mucus and plasma were measured in specimens collected at baseline from 156–169 of the participants. In preparation for analysis, to normalize the distribution of antibody optical density units, the data were log transformed. Levels of IgG to EB (r = 0.23; P < .004) and of IgG to CHSP60 (r = 0.25; P < .002) were highly correlated between endocervical mucus and plasma (data not shown). In univariate and multivariate analyses, after adjustment for age, years lived in Nairobi, and being single, levels of IgA and IgG detected in endocervical mucus and plasma were not significantly associated with an altered risk of

Table 3. Initial laboratory values in a cohort of commercial sex workers in Nairobi, Kenya.

Category	Value	Valid no. of participants
Cell count, mean ± SD		
White blood cell, log cells/μL	5.9 ± 1.8	294
$CD4^+$, cells/μL	864 ± 354	294
$CD8^+$, cells/μL	733 ± 373	294
Antibody levels in endocervical mucus, mean ± SD, log ODUs		
IgA to EB	−0.67 ± 0.58	159
IgG to EB	−0.49 ± 0.47	159
IgA to CHSP60	−1.22 ± 0.70	159
IgG to CHSP60	−0.64 ± 0.52	156
Antibody levels in plasma, mean ± SD, log ODUs		
IgG to EB	−0.57 ± 0.39	169
IgG to CHSP60	−0.74 ± 0.52	169
Cytokine production by PBMCs after stimulation with antigen		
Positive for IFN-γ		
EB	69 (40)	173
CHSP60	29 (18)	161
Positive for IL-10		
EB	119 (75)	158
CHSP60	119 (78)	153
Positive for IL-13		
EB	59 (35)	171
CHSP60	19 (12)	160
Positive for IL-5		
EB	50 (33)	150
CHSP60	17 (13)	130

NOTE. Data are no. (%) of participants, unless otherwise noted. CHSP60, chlamydia heat-shock protein 60; EB, chlamydia elementary body; IFN, interferon; IL, interleukin; ODUs, optical density units; PBMCs, peripheral-blood mononuclear cells.

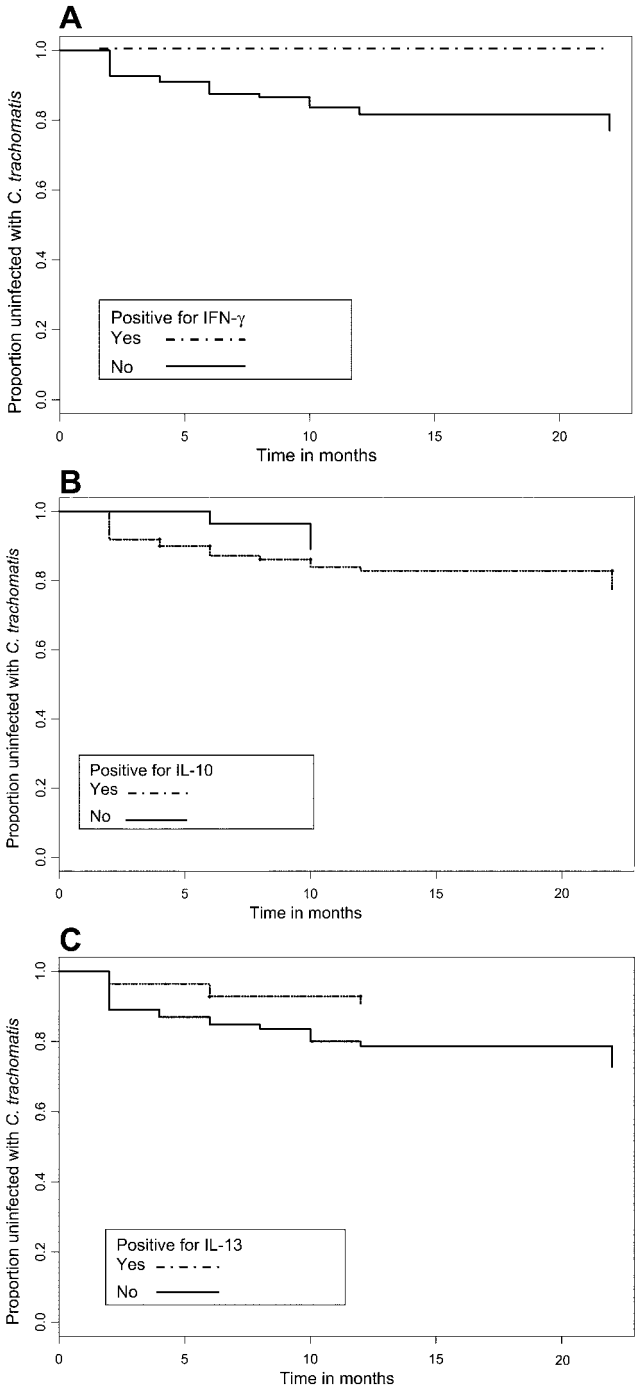

Figure 2. Comparison of time to first *Chlamydia trachomatis* infection in women with and without an interferon (IFN)–γ response by peripheral-blood mononuclear cells (PBMCs) stimulated with chlamydia heat-shock protein (CHSP) 60 *(A);* an interleukin (IL)–10 response by PBMCs stimulated with CHSP60 *(B);* and an IL-13 response by PBMCs stimulated with chlamydia elementary body *(C).*

incident *C. trachomatis* infection (table 4). Furthermore, levels of IgG and IgA to EB and CHSP60 did not correlate with levels of cytokines produced by PBMCs after stimulation with EB and CHSP60 (data not shown; $P > .05$, for all).

DISCUSSION

The data presented here are among the strongest to support a direct role for *C. trachomatis*–specific adaptive immune responses in reducing the risk of human chlamydia infection. The study demonstrated that a Th1 (IFN-γ) response to CHSP60 was associated with protection against *C. trachomatis* infection, whereas an IL-10 response to the same antigen appeared to increase the risk of infection. Interestingly, EB-induced production of IL-13 by PBMCs also correlated with a reduced risk of genital-tract infection.

The finding that both younger age and fewer years of prostitution correlated with an increased risk of *C. trachomatis* infection is consistent with the hypothesis of the development of immunity. Two of the strongest epidemiological risk factors for *C. trachomatis* infection were incident *N. gonorrhoeae* infection during follow-up and *C. trachomatis* infection at enrollment. Although gonococcal infection is a well-established risk factor for chlamydia infection, the mechanistic basis for this association is unknown [27], but it may be related to T cell inactivation by gonorrhea [28]. The absence of an association between HIV-1 serostatus and the risk of *C. trachomatis* infection is consistent with the findings of other recent investigations [29, 30].

It is of interest that the immunobiological response to chlamydia infection was more readily evident when T cell responses to specific antigens, rather than whole EBs, were assayed. This was previously found in an immunoepidemiological study of trachoma, in which individuals with severe trachomatous scarring had PBMCs that produced IL-4 to CHSP60 more frequently than did PBMCs from matched community control subjects without scarring [25]. In the present study, PBMCs from a greater proportion of participants produced IFN-γ after stimulation with EB (40%) than with CHSP60 (18%), but only the latter was associated with protection. The mechanism behind this finding is not clear, but the result could indicate that EBs contain multiple T cell antigens, only some of which elicit protective responses. Chlamydia encodes 894 proteins [31], and only a small subset of these are known to be T cell antigens [12]. Future studies on the immune correlates of protection should focus on antigen-specific immune responses by using additional immunologically relevant proteins.

The observation that immune responses to CHSP60 correlate with chlamydia immunobiology is consistent with previous observations. Serological data have consistently demonstrated a strong association between antibody responses to CHSP60 and complications of *C. trachomatis* infection, including PID, tubal infertility, ectopic pregnancy, and scarring trachoma [26, 32–35]. In addition to the study of trachoma that demonstrated that CHSP60-induced production of IL-4 by PBMCs correlated with scarring disease [25], a study of T cell clones from women with tubal infertility demonstrated that they commonly pro-

Table 4. Hazard ratios (HRs) and 95% confidence intervals (CIs) from a Cox proportional hazards model to test for associations between incident *Chlamydia trachomatis* infection and (1) levels of antibodies to chlamydia elementary body (EB) and chlamydia heat-shock protein 60 (CHSP60) and (2) cytokine response by peripheral-blood mononuclear cells (PBMCs) after stimulation with chlamydia antigens in a cohort of commercial sex workers in Nairobi, Kenya.

	Unadjusted		Adjusted[a]	
Category	HR (95% CI)	*P*	HR (95% CI)	*P*
Antibody levels in endocervical mucus				
IgA to EB	1.1 (0.6–2.3)	.7	1.8 (0.7–4.5)	.2
IgG to EB	1.9 (0.5–7.3)	.4	2.4 (0.5–11.1)	.3
IgA to CHSP60	0.6 (0.3–1.2)	.2	0.8 (0.3–2.3)	.7
IgG to CHSP60	0.8 (0.4–1.6)	.5	0.8 (0.4–1.9)	.7
Antibody levels in plasma				
IgG to EB	0.6 (0.1–3.4)	.6	0.7 (0.2–3.4)	.7
IgG to CHSP60	0.2 (0.1–0.7)	.1	0.4 (0.1–1.2)	.09
Cytokine production by PBMCs after stimulation with antigen				
Positive for IFN-γ				
EB	1.0 (0.4–2.9)	1.0	1.0 (0.4–2.8)	1.0
CHSP60[b]	0.2 (0.03–1.0)	<.05	0.2 (0.02–1.0)	<.05
Positive for IL-10				
EB	1.4 (0.5–4.6)	.5	1.7 (0.5–5.8)	.4
CHSP60	6.7 (1.0–47.1)	.06	5.3 (0.8–34.5)	.08
Positive for IL-13				
EB	0.2 (0.1–0.7)	.01	0.2 (0.1–0.8)	.02
CHSP60	0.4 (0.1–2.4)	.3	0.3 (0.1–1.8)	.2
Positive for IL-5				
EB	0.3 (0.1–1.1)	.07	0.3 (0.1–1.0)	.06
CHSP60	1.0 (0.3–3.6)	1.0	0.8 (0.2–3.3)	.7

NOTE. IFN, interferon; IL, interleukin.

[a] Adjusted for the following significant demographic factors: age, years lived in Nairobi, and being single (never married).

[b] No participant with a positive IFN-γ PBMC response to CHSP60 had an incident *C. trachomatis* infection. Therefore, to fulfill the requirements of survival analysis—which does not allow for zero events—a single participant was switched to infection with *C. trachomatis*. Thus, these results likely represent an underestimation of the HR (95% CI) and *P* value.

duced IL-10 in response to CHSP60 [26]. Recently, Debattista et al. [24] reported that women with chlamydia PID or a history of repeated *C. trachomatis* infection had PBMCs that produced less IFN-γ in response to CHSP60 than did women in a variety of comparison groups, including women with a single episode of *C. trachomatis* infection. The present study showed that women with IFN-γ responses to CHSP60 stimulation had a substantially reduced risk of chlamydia infection over the course of 20 months of exposure. In fact, no woman with an IFN-γ response to CHSP60 became infected during follow-up. IL-10 responses to CHSP60, on the other hand, were associated with a 5-fold increased risk of infection, although the association did not reach statistical significance. In murine models of chlamydia infection, IL-10 has clear detrimental effects on host resistance and clearance [12, 36], and the present study suggests that IL-10 may also have detrimental effects on human resistance to infection. The cellular source for IL-10 was not defined in this study, and, although IL-10 can be produced by Th2 cytokine–producing cells, it can also be produced by other cell types, such as $CD4^+CD25^+$ regulatory T cells. These cells account for 5%–10% of circulating peripheral-blood leukocytes, recognize self-antigen, and are known to regulate immunity to intracellular pathogens, such as *Leishmania* species [35]. Thus, it may be that CHSP60, which has both self- and chlamydia-specific epitopes [36], differentially engages $CD4^+$ Th1 cells and $CD4^+CD25^+$ regulatory T cells at the site of challenge infection, thereby determining susceptibility and resistance to *C. trachomatis* infection.

Why certain individuals develop protective immunity against *C. trachomatis* infection and others do not remains unknown. HLA class II alleles may present different chlamydial peptides that evoke damaging, protective, or regulatory immune responses by $CD4^+$ T cells, and cytokine polymorphisms may alter the risk of disease [37–39]. The risk of *C. trachomatis*–associated tubal infertility and trachoma has been associated with unique HLA class II alleles, and the HLA-DQA*0401/DQB1*0402 heterodimer has been associated with increased levels of antibodies to CHSP60 [32]. Among women with chlamydia-related tubal infertility, T cell responses to CHSP60 were associated with a specific IL-10 promoter polymorphism (IL-10–1082AA) and with specific HLA class II DQ alleles (HLA-DQA1*0102 and HLA-DQB1*0602) [39]. Thus, genetic factors that regulate the induction and ac-

tivation of Th1 and $CD4^+CD25^+$ regulatory T cells (perhaps via dendritic cells) may be critical in directing immunity or immunopathological resistance against *C. trachomatis*.

The observation that EB-induced production of IL-13 by PBMCs correlated with protection against chlamydia infection was unexpected. Along with IL-4, IL-13 has been categorized as a Th2 cytokine [40]. However, there are significant differences between IL-13 and IL-4 with respect to certain functions—such as regulation of Th2 and Th1 polarization—because T cells do not express type II IL-4R and, therefore, do not respond to IL-13. This suggests that IL-13 does not cross-regulate $CD4^+$ T cell polarization [40]. IL-13 is essential to cell-mediated immunity against *Listeria monocytogenes,* in part by activating the production of IL-12 by host macrophages [41]. Furthermore, IL-13 induces the migration and maturation of dendritic cells, and both IL-12 and mature dendritic cells are known to be essential to the development of chlamydia immunity [42, 43]. Because IL-13 is also known to activate tissue fibrosis [44], its production may also be relevant to the fibrotic sequelae of chlamydia infection, such as infertility and blindness.

We failed to find a correlation between local and systemic levels of chlamydia antibodies and the risk of reinfection. Nevertheless, in the murine model of chlamydia infection, it has been shown that B cells remain necessary for host resistance [8], although T cell activation is more important for immunity [11]. Earlier studies in humans demonstrated an inverse relationship between levels of IgA in cervical secretions and the quantity of *C. trachomatis* shed in the genital tract [44]. In addition to the possibility that they play roles in neutralizing infectious organisms, antibodies to *C. trachomatis* surface structures are known to dramatically enhance opsonization of dendritic cells and to promote strong Th1 immune responses [43]. The failure to observe an association between antibody responses and chlamydia immunity in the present study may reflect either the absence of an association, the limitations of the serological assays used, or confounding between cellular and humoral mechanisms of protection. However, on the basis of available data, levels of antibodies to EB and CHSP60 do not appear to be good proxies for immunity after experimental vaccination.

Although the present study has demonstrated that production of IFN-γ by PBMCs stimulated with CHSP60 correlates with protection against infection in humans, this finding should not be overinterpreted. It remains possible that a pathway other than IFN-γ is involved—for example, induction of other effectors via IL-12–dependent immunity or $CD8^+$ T cells [8, 20, 45–47]. Furthermore, the validity of the use of CHSP60 as a vaccine antigen is uncertain: use of CHSP60 as a recombinant protein vaccine in the guinea pig model of ocular infection was not protective [48], although use of CHSP60 as a DNA vaccine in a mouse model of *Chlamydia pneumoniae* lung infection was protective [49]. Additionally, the type of cytokine response elicited by CHSP60 appears to be critical to protection; thus, if CHSP60 is to be evaluated as a vaccine candidate, it must be carefully delivered to polarize a Th1 IFN-γ response. Further study of this and other chlamydial antigens and of their immune correlates of protection in immunoepidemiological cohort investigations—especially ones that include sexually active adolescents—are highly desirable. Study designs could incorporate cohorts of commercial sex workers, such as that undertaken here, or communities in which trachoma is hyperendemic, where the incidence of reinfection following mass treatment has approached an astounding rate of 12.3% per month [50]. In either situation, it should be feasible to correlate baseline immunological measurements with the risk of *C. trachomatis* infection, because of the high risk of reinfection. Such data will considerably advance *C. trachomatis* vaccine development by establishing robust correlates of protective immunity.

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