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Anti-chlamydia IgG and IgA are insufficient to prevent endometrial Chlamydia infection in women and increased anti-chlamydia IgG is associated with enhanced risk for incident infection

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Abstract

Problem: Chlamydia infections in women can ascend to the upper genital tract, and repeated infections are common, placing women at risk for sequelae. The protective role of anti-chlamydia antibodies to surface exposed antigens in ascending and incident infection is unclear.

Method of Study: A whole-bacterial ELISA was used to quantify chlamydia-specific IgG and IgA in serum and cervical secretions of 151 high-risk women followed longitudinally. Correlations were determined between antibody and cervical burden and causal mediation analysis investigated the effect of antibody on ascension. We examined the relationship of antibody to incident infection using the marginal Cox model.

Results: Serum and cervical anti-chlamydia IgG and cervical IgA levels correlated inversely with cervical burden. While lower burden was associated with reduced ascension, causal mediation analysis revealed that the indirect effects of antibody mediated through reductions in bacterial

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burden were insufficient to prevent ascension. Analysis of women uninfected at enrollment revealed that serum and cervical anti-chlamydia IgG were associated with increased risk of incident infection; hazard ratio increased 3.6-fold (95% CI, 1.3 – 10.3), and 22.6-fold (95% CI, 3.1 – 165.2) with each unit of serum and cervical IgG, respectively.

Conclusions: Although anti-chlamydia IgG and IgA correlated with reduced cervical chlamydia burden, they failed to prevent ascension and increased levels of anti-chlamydia IgG were associated with increased risk for incident infection.

Keywords

chlamydia; women; antibody; genital tract; endometrium; incident infection; causal mediation

INTRODUCTION

Chlamydia trachomatis is highly prevalent due to its asymptomatic nature, and its ability to evade immunity results in chronic and repeated infections that can lead to serious reproductive sequelae in women. Chlamydia elementary bodies (EBs) rapidly invade host epithelial cells through multiple mechanisms¹⁻⁴, where they transform to reticulate bodies and replicate within a protective vacuole (inclusion) before re-differentiating to infectious EBs that escape to infect adjacent cells and hosts exposed through sexual contact. CD4 T cell mediated immunity is essential for host defense against chlamydia and plays a key role in eradication of infection and resistance to reinfection⁵⁻⁸. In contrast, a protective role for antibody in chlamydial immunity is unresolved.

Anti-chlamydia antibodies against surface exposed proteins could neutralize infectious extracellular EBs prior to host cell invasion, and thereby prevent infection or reduce infectious burden. Antibody-mediated chlamydia neutralization has been demonstrated *in vitro*⁹⁻¹¹, but animal studies suggesting a protective role for this mechanism are limited^{12,13}. Other studies support a role for antibody in limiting infection by enhancement of chlamydia opsonophagocytosis and degradation^{14,15}, but this process appears dependent on T-cell interferon- γ (IFN- γ) production¹⁵.

In two longitudinal analyses of highly exposed women, chlamydia-specific CD4 T cell IFN- γ responses, and not serum anti-chlamydia IgG titers, were associated with a reduced risk of reinfection^{16,17}. Same serovar repeated infections are frequent^{18,19}, and high titers of anti-chlamydia IgG antibodies are associated with enhanced disease²⁰⁻²². These findings suggest high IgG titers are a marker of repeated and/or prolonged chlamydia exposure, and do not protect from reinfection.

We previously investigated the role for anti-chlamydia EB IgG in limiting chlamydial infection¹⁶. We observed both that serum titers of anti-EB IgG were associated with reduced cervical burden, and that women with lower cervical burden were at reduced risk for endometrial infection²³. Paradoxically, anti-EB IgG titers failed to correlate with absence of endometrial infection¹⁶. These data suggest anti-EB IgG antibodies are ineffective at limiting ascension and its associated risk for disease. However, these studies did not investigate mucosal IgG, or IgA, which may exhibit more potent, neutralizing effects²⁴.

Brunham et al. reported that anti-chlamydia IgA, but not IgG, was more frequently detected in genital secretions of women who did not develop salpingitis, compared to those who did, suggesting a role for local IgA in limiting ascension²⁵. Levels of HIV specific mucosal IgA were shown to correlate with resistance to HIV infection in Kenyan sex workers²⁶, and IgA was shown to act through both extracellular neutralization and intracellular viral inhibition after transcytosis^{27,28}.

Despite these protective effects, some antibodies can also mediate enhanced uptake of pathogens. Antibody mediated enhancement of infection has been well described for viruses^{29,30}. In addition, IgG specific to the major outer membrane protein (MOMP) of chlamydia has been shown to enhance chlamydial uptake and transcytosis through polarized genital tract epithelial cells, and increase murine genital tract infection¹¹. To explore the potential that direct effects of antibody might counteract limitation of ascension mediated through lowering cervical burden, we performed a causal mediation analysis. We determined that the probability of endometrial infection was significantly reduced by both serum anti-EB IgG and cervical anti-EB IgA mediated through effects on burden. However, the burden-independent effects of antibody on ascension negated these protective effects, leading to an overall effect that was neither protective or detrimental. We also determined that both serum and cervical anti-EB IgG correlated with significantly increased, rather than decreased risk, of repeat infection. These novel results indicate that natural infection induces high levels of antibody to surface exposed chlamydial EB proteins in women that can effectively lower cervical burden, but are insufficient to prevent endometrial infection or reinfection and serve as biomarkers of enhanced susceptibility.

METHODS

Patient Population

The Institutional Review Boards for Human Subject Research at the University of Pittsburgh and the University of North Carolina approved the study and all participants provided written informed consent prior to inclusion. Samples were collected from participants recruited into a previously described T cell Response Against Chlamydia (TRAC) cohort¹⁶, which enrolled 246 asymptomatic women (age 15–30 years) at high risk for sexually transmitted infection from three urban sites in Pittsburgh, Pennsylvania from 2011–2015. Eligibility criteria included clinical evidence of mucopurulent cervicitis, diagnosis of chlamydia prior to treatment, or reported sexual contact with a man recently diagnosed with chlamydia urethritis or nongonococcal urethritis. Women diagnosed with pelvic inflammatory disease or who were pregnant were excluded.

Demographic and clinical data were obtained using a standardized questionnaire and examination. Cervical secretions were adsorbed onto an ophthalmic sponge (Beaver-Visitec, Waltham, MA) placed within the endocervix (30 seconds) of each patient recruited into the TRAC cohort. An endometrial biopsy was then obtained to determine extent of infection. Cervical and endometrial swab samples were tested for *C. trachomatis*, *N. gonorrhoeae*, and *M. genitalium* using nucleic acid amplification tests (NAAT), (Aptima Combo 2; Gen-Probe, and APTIMA MG; Gen-Probe) as described previously¹⁶. Cervical chlamydia burden was estimated via quantitative PCR using genomic DNA extracted from reserved cervical swab

eluates as template²³. Women with positive cervical and endometrial Chlamydia NAATs were designated Endo+, while those with a positive cervical NAAT only were defined as Endo-. Serum was collected for antibody assays. All women were treated with ceftriaxone and azithromycin at enrollment regardless of infection status. Participants returned for follow-up visits at 1, 4, 8, and 12 months after enrollment, microbiologic, medical, sexual history and exposure, and contraception data were gathered at each follow-up visit, and all participants that tested positive for chlamydia were treated with azithromycin.

Antibodies in Serum and Cervical Secretions

Cervical secretions were eluted from sponges as previously described³¹. IgG and IgA assays were performed on cervical secretions and serum samples collected from the first 151 participants enrolled into the TRAC cohort. Concentrations of anti-EB IgG and IgA were measured using a whole-EB ELISA that detects chlamydial outer membrane and periplasmic proteins, but not intracellular proteins³¹. Concentrations of total IgG and IgA in serum and secretions were measured using a previously described ELISA which readily detects secretory IgA in mucosal secretions³². Concentrations of anti-EB IgG or IgA antibody in each sample were determined from standard curves that consisted of pooled human serum from CT-infected subjects, then divided by the concentration of total IgG or IgA to obtain the specific activity. Non-specific antibody values were quantitated using 2 IgG and 9 IgA myeloma antibodies as well as anti-HIV-1 gp120 and gp41 human IgG1 mAbs derived from transformed human B cells or hybridomas as previously described³¹. The cut-off for detection of a specific anti-EB antibody response was defined by a value that was $>$ the mean + 3 standard deviations (SD) of the non-specific antibody values, which implies a value greater than 99% of the non-specific antibody values.

Statistical Analysis

Anti-EB antibody and cervical chlamydia burden values were log transformed and tested for normal distribution using the Shapiro–Wilk normality tests. Pearson correlation was used to measure the strength of association of the four antibodies to each other, and to cervical burden. A comparison of cervical chlamydial burden in Endo+ versus Endo- women was conducted by unpaired *t* test.

Antibody and ascension by causal mediation analysis.—We considered ascending infection (Endo+ vs. Endo-) as a binary outcome, and we defined chlamydia cervical burden as a mediator, and antibody as an exposure. These definitions were based on prior data from this cohort that revealed women with lower cervical burden had higher titers of serum anti-chlamydia IgG, implying antibody drives a reduction in burden¹⁶. This led us to use a well-established causal mediation algorithm published by Imai and Tingley³³ to investigate the association of antibody with endometrial infection. Using causal mediation, the relationship between antibody and ascension is decomposed into a direct link and an indirect link. The causal mediation effect represents the indirect effect of the exposure (antibody) on the outcome (ascension) through the mediating variable (bacterial burden). Whereas, the direct effect is the effect of the exposure (antibody) on the outcome (ascension) that is independent of the mediator (burden). The total effect is the sum of the indirect and direct effects (Figure 1). The Imai and Tingley algorithm models the exposure as binary and two levels of

exposure can be accommodated. For this analysis, we compared the median antibody level of participants with EB-specific responses versus the median antibody level of participants with responses below the cut-off for EB antibody specificity to determine if detection of an EB-specific antibody response was associated with reduced endometrial infection. Since we previously determined that oral contraceptive pill (OCP) use and *N. gonorrhoeae* co-infection were significant risk factors for ascending infection¹⁶, we treated these as confounders.

Mediation processes are framed in terms of intermediate variables between an independent variable and a dependent variable, where t is the independent variable (antibody), Y is the dependent variable (ascension), and M is the mediator variable (cervical chlamydial burden) that is supposed to transmit the causal effect of t to Y ³³. The total effect of t on Y is referred to as the total effect (TE), and that effect is then partitioned into a combination of a direct effect (DE) of t on Y , and an indirect effect (IE) of t on Y that is transmitted through M .

We defined the causal mediation effect of antibody as follows:

$$\delta_i(t) = E[Y_i(t, M_i(1)) - Y_i(t, M_i(0))]$$

Y_i denotes the outcome (ascension) for subject i ; t is the exposure (antibody) status, $t=0$ denotes absence of EB-specific response, $t=1$ denotes presence of EB-specific response; E denotes expected value. This determines what change would occur to the outcome, if one changes the mediator (cervical chlamydia burden) value that would be realized under the presence of an EB-specific response, $M_i(1)$, to the value that would be observed under the absence of an EB-specific response, $M_i(0)$, while holding the exposure status constant. If antibody has no effect on cervical chlamydia burden, $M(1)=M(0)$, then the causal mediation effect is zero.

Depending on level of t , we have:

$$\delta_i(1) = E[Y_i(1, M_i(1)) - Y_i(1, M_i(0))]$$

$$\delta_i(0) = E[Y_i(0, M_i(1)) - Y_i(0, M_i(0))]$$

In causal mediation analysis, we are interested in the average causal mediation effect between exposed (median of participants with an EB-specific response) and control (median of participants without an EB-specific response) populations:

$$\delta^- = \frac{1}{2}(\delta(1) + \delta(0))$$

The direct effect represents the effect of the exposure on outcome while holding the level of the mediator constant. We defined the direct effect as follows:

$$\zeta_i(t) = E[Y_i(1, M_i(t)) - Y_i(0, M_i(t))]$$

For $t=0,1$. For example, $\zeta_i(1)$ represents the direct effect of the presence of an EB-specific response on subject i 's ascension outcome while holding the chlamydia burden level constant. Similarly, averaging over the relevant population, we can define the average direct effects.

To investigate whether the effect of anti-EB antibody on burden is different in women with EB-specific antibody responses and those without, we used statistical software R mediation package (Version 4.4.6) for biostatistical interactions. A key assumption of mediation is that no unmeasured factors confound the relation between mediator and outcome. To investigate for unobserved confounder bias in our mediation analysis, we performed a sensitivity analysis that quantified the degree to which unmeasured confounders must be present to reverse the direction of the mediation effect. If the inference of a causal mediation effect is sensitive, a slight violation of the assumption (small ρ value) may lead to a substantially different conclusion (reverse direction of effect). The null hypothesis is that unexplained variances in mediator and outcomes are independent, and therefore the sensitivity parameter, correlation (ρ), between these two variances should be zero. Results of this sensitivity analysis shows how much the casual mediation effect changes as a function of ρ . We used Imai and Tingley algorithms and statistical software R package mediation for both mediation and sensitivity analyses^{33–35}.

Antibody and incident infection.—Since active infection drives antibody production we stratified our analysis into separate evaluations of uninfected and infected women. We used the non-parametric *Mann–Whitney U test* to determine if antibody levels differed among women who developed incident infection during a year of follow-up versus those who did not. Women who tested positive for chlamydia infection at any follow-up visit were treated with azithromycin. These women were assumed to be cured of their infection and at risk for repeat infection. We therefore proceeded to determine the relative hazard ratios (HR) for antibody effects on incident infection using the Wei–Lin–Weissfeld method, a marginal Cox model that accounts for multiple chlamydial infections per person and adjusts for within-subject correlations³⁶. Adjustments were made for previously identified risk factors associated with incident infection, including age, current or incident gonorrhea, chlamydia infection at enrollment; and sex with new, uncircumcised, or infected partners¹⁶. To formally test situations in which the antibody effect differs according to infection status (Uninfected, Endo-, and Endo+), we added an interaction term of antibody \times infection status at enrollment to the model. This enabled a single test of antibody effects in the entire group rather than performing separate tests in the three groups; these analyses were performed in SAS 9.4 (SAS Institute Inc.). All analyses used a P value of < 0.05 as significant.

RESULTS

Baseline Characteristics of Participants

Data from the first 151 women recruited into TRAC, whose anti-EB IgG, and anti-EB IgA had been quantified in serum and cervical secretions via ELISA, were analyzed in this study. Among them, 56 (37%) were negative for chlamydia infection at cervix and endometrium, 56 (37%) had cervical infection only (Endo-), and 39 (26%) had cervical and endometrial

infection (Endo+). Consistent with our previous report for 225 women of this cohort, women in this study were young (median age 21 years; range, 18–35 years), single (89%), and African American (66%) (Supplementary Table 1). The majority (57%) reported a previous diagnosis of chlamydial infection, and (25%) reported having had 2 prior infections. About 68% of participants reported a past history of at least one bacterial STI such as *N. gonorrhoeae* (23%) and/or *T. vaginalis* (27%). 117 women (77%) completed at least 3 follow-up visits, and 87 (58%) completed 4. There were no sociodemographic differences between women who attended all visits and those who did not (data not shown).

Relationship between Anti-chlamydia EB Antibodies and Ascension

We determined anti-EB IgG and IgA antibody levels in serum and cervical secretions to explore both systemic and mucosal responses to multiple outer membrane components of the infectious developmental form of chlamydia³¹. We first determined the strength of relationships among the anti-EB antibodies measured at enrollment. Although all antibody relationships were significant and positively correlated, only the correlation between serum and cervical anti-EB IgG levels were strong ($R=0.57$, $P=1.82E-13$). The positive correlations were weak to moderate for serum and cervical anti-EB IgA levels ($R=0.26$, $P=0.002$), serum anti-EB IgG and IgA levels ($R=0.26$, $P=0.0013$), and cervical anti-EB IgG and IgA levels ($R=0.36$, $P=1.18E-5$).

We next determined the relationship of the various antibody subtypes to cervical chlamydia load. Inverse correlations were significant for both serum and cervical anti-EB IgG and cervical chlamydia burden, with the strength of the inverse correlation being stronger for serum anti-EB IgG compared to cervical anti-EB IgG ($R=-0.41$, $P=4.20E-5$ and $R=-0.20$, $P=0.05$ respectively). A weak inverse correlation of borderline significance was observed between anti-EB IgA in cervical secretions and cervical chlamydia load ($R=-0.29$, $P=0.01$), and a non-significant weak inverse correlation was observed between serum anti-EB IgA levels and chlamydia burden ($R=-0.14$, $P=0.17$) (Figure 2).

We next investigated the association between chlamydia load and ascension. Cervical burden was significantly lower in 56 women with cervical infection only, compared to 39 women with endometrial infection ($P=3.51E-08$, t test) (Supplementary Figure 1), consistent with prior findings within the cohort²³. Finally, we performed a causal mediation analysis to test whether antibody impacted ascension (Figure 1). Since inverse correlations were determined between each antibody subtype and cervical chlamydia burden, we delineated the effects of antibody subtypes on ascension that are mediated through their effects on burden (indirect), as well as effects that are independent of their effects on pathogen load (direct) (Figure 1A). The combination of burden/mediator-dependent, indirect effects and burden-independent, direct effects comprises the total effect (Figure 1B). The results are listed in Table 1 and graphically displayed in Figure 3. The antibody effect mediated through reduced burden was a decreased probability of ascension. This was significant for anti-EB serum IgG and cervical IgA, borderline for cervical IgG, and non-significant for serum IgA (Table 1). The direct effects independent of burden negated the protective role of antibodies, leading to a non-significant relationship between serum and mucosal anti-EB IgG and serum and mucosal anti-EB IgA on the probability of endometrial infection.

A sensitivity analysis determined that the observed mediated effect was unlikely to be caused by an unmeasured confounder for all antibody subtypes assessed (Supplementary Figure 2). These analyses strengthen the validity of the results of causal mediation. To exclude the possibility that the effects of antibody on burden differed between women with EB-specific antibody responses and those without, we used R mediation analysis to detect biostatistical interactions of antibody and pathogen load and determined that no interaction existed for any antibody subtype ($P=0.93$, 0.99 , 0.51 , and 0.80 for serum IgG and IgA, cervix IgG and IgA respectively).

Relationship of Anti-chlamydia EB Antibodies with Incident Infection

We next examined the relationship between anti-EB antibodies and incident infection. Recognizing that active infection drives induction of antibody, we stratified our analysis of incident infection into two separate evaluations of uninfected and infected women to determine if baseline antibody levels altered incident infection risk. Anti-EB serum and cervical IgG were higher in uninfected women who experienced incident infection(s), compared to those that did not (Figure 4A-B and Table 2). Although this relationship was not observed with anti-EB serum and cervical IgA levels, higher levels of mucosal anti-EB IgA clearly failed to prevent infection (Figure 4C-D and Table 2). After determining that baseline anti-EB IgG levels varied between uninfected women who became infected versus those who did not, we formally tested the association of anti-EB IgG levels with increased risk of incident chlamydia infection and adjusted for 6 potential confounders: age, gonorrhea, chlamydia infection at enrollment, sex with new, uncircumcised, or infected partners (Table 2)¹⁶. Each unit of anti-EB serum IgG significantly increased the HR of incident infection 3.63-fold (95% CI, 1.28 – 10.34). Cervical anti-EB IgG was even more pronounced at 22.57-fold (95% CI, 3.08 – 165.16).

In contrast, IgG antibody levels at baseline did not predict an altered risk of incident infection in Endo- or Endo+ infected women, with statistical differences being observed between the HR for uninfected women and those who were Endo- and Endo+ ($P=0.044$ and 0.026 for serum anti-EB IgG; and $P=0.001$ and 0.004 for cervical anti-EB IgG respectively). Baseline levels of IgA antibodies in infected women were not associated with altered risk of incident infection (Table 2 and Supplementary Figure 3).

DISCUSSION

Chlamydial infection reaching the upper genital tract of women increases risk for tubal factor infertility and chronic pelvic pain. A chlamydia vaccine that induces complete immunity to infection would be optimal, but a vaccine that prevents ascension could eliminate these morbidities. Thus, defining immune responses that limit chlamydial ascension is important for vaccine development. Prior analysis of a subset of infected women in the TRAC cohort revealed that Endo- women had a lower mean cervical chlamydia burden when compared to Endo+ women, and that higher titers of serum anti-EB IgG correlated inversely with cervical burden²³. However, we did not detect any relationship between anti-EB IgG levels and ascension¹⁶. In this study, we confirmed higher cervical chlamydia burdens were present in Endo+ women, that serum and mucosal levels of anti-EB

IgG were strongly correlated with each other, and that both were inversely correlated with pathogen load. Since cervical IgG is a composite of IgG transported from the serum into cervical secretions^{37,38} and IgG produced by local plasma cells, it is logical that anti-EB IgG levels in serum and cervical secretions were highly correlated and that both were associated with reduced burden. The correlation between serum and cervical anti-EB IgA levels was weakly positive, and although both correlated inversely with burden, the correlation was weak and not significant for serum IgA; this can be explained by the fact that mucosal IgA is locally produced by IgA-producing plasma cells.

The inverse relationships of antibody with burden and burden with ascension suggests that higher levels of antibody should be associated with reduced risk of ascension. However, causal mediation analysis did not detect a significant relationship between any antibody subtype and ascension when the total effect was determined. Examination of the indirect effects of antibody on ascension, mediated through alterations in cervical burden, revealed that both serum anti-EB IgG and cervical anti-EB IgA were associated with a significantly decreased probability of ascension. This contrasts with burden-independent effects, which, while not significant, abrogated the protective effects of antibody mediated through burden. These findings suggest the possibility for antibody-mediated enhanced uptake of chlamydia that has been well-described for non-neutralizing antibodies in viral infections^{29,30}. Evidence that antibody might promote chlamydial infection directly via transcytosis has been obtained in cell culture and *in vivo*. Anti-IgG antibody against chlamydial MOMP enhanced neonatal receptor FcR (FcRn)-dependent uptake and translocation of chlamydia in polarized epididymal epithelia grown on transwells, and female mice challenged with chlamydia opsonized with anti-MOMP IgG displayed delayed infection clearance and exacerbated oviduct disease¹¹. Prolonged infection is likely to result in higher titers of serum and mucosal anti-EB antibodies, elicited by continued antigen exposure. An ineffectual T cell response would result in prolonged infection. Thus, detection of high levels of anti-EB antibodies may reflect poor development of protective T cell immunity and serve as a marker of increased risk or susceptibility. Baseline levels of systemic and mucosal anti-EB IgG in women uninfected at enrollment correlated with a significantly enhanced risk of incident infection which is further evidence that elevated antibodies of this isotype serve as a marker of inferior anti-chlamydial immunity. These data are in stark contrast to results from this same cohort that revealed significant increases in frequencies of peripheral blood CD4 T cell IFN- γ responses to chlamydial proteins were associated with protection from incident infection²³.

Although we did not examine IgG subtypes associated with Th1 versus Th2 responses, given the considerable data related to the protective effects of Th1 responses and the detrimental effects of Th2 responses on chlamydia infection^{5-8,39-41}, it is plausible that the subset of women with the highest anti-EB IgG responses are Th2-skewed. This would have resulted in an inadequate IFN- γ response with high amounts of anti-chlamydial antibody at the site of infectious challenge. Naglak et al. showed that IFN- γ was necessary for antibody-mediated opsonophagocytosis and enhancement of chlamydia killing via neutrophils¹⁵. Reduced neutrophil opsonophagocytosis and killing of infectious EBs would promote enhanced susceptibility to infection. Determination of the subclass of anti-chlamydia serum and mucosal antibodies could aid in determining the polarization of the immune response and

the relative contribution of serum-transported versus locally-produced antibodies. The absence of any correlation between levels of anti-EB antibodies present at enrollment and incident infection rate in infected women is likely due to the strong influence of active infection on antibody production. This would mask any Th2 versus Th1 bias, preventing detection of Th2-prone women with an increased risk of incident infection.

Strengths of our study include rigorous biostatistical methods, including mediation analysis that determined the mechanism of effects of anti-EB antibodies on ascending infection by dissecting them into mediator-dependent and -independent effects; appropriate adjustments for known behavioral and clinical characteristics that influence infection; and a sensitivity analysis that revealed a low likelihood of unmeasured confounders influencing the observed causal mediator effect. An important limitation is the lack of information regarding specificity of serum- and secretion-derived anti-chlamydial antibodies. EB antibody ELISA detects antigens exposed on the surface of EBs, such as MOMP, but not cytoplasmic or inclusion membrane chlamydia antigens³¹. The latter may have a protective role; e.g., IgG specific for InCA, a chlamydia inclusion membrane protein can be transported intracellularly by FcRn, resulting in recruitment of autophagic proteins and reduced infection¹¹. Other inclusion membrane proteins that are cytoplasmic facing can be bound by intracellular IgG, with resulting reductions in chlamydial progeny⁴². Although our analyses indicated that the total effect of serum or mucosal anti-EB antibodies were insufficient to protect from ascension or incident infection, it is possible that antibody to specific chlamydia antigens could have a protective effect. Although labor intensive, chlamydial protein and peptide libraries^{43–46}, in combination with isotype analysis could be used to correlate chlamydia protein-specific antibody responses with endometrial ascension and rates of incident infection in highly exposed individuals. Detection of chlamydial antigens associated with protection against ascension or reinfection would identify vaccine candidates.

Our failure to detect correlations of antibody levels to surface exposed chlamydial proteins with protection from ascension or from incident infection in women naturally infected with chlamydia does not indicate vaccines that incorporate these antigens will be ineffective. Data from murine models indicate significant, although not complete, protection can be induced by vaccination with intact EBs or chlamydia MOMP, when administered with Th1-inducing adjuvants^{47–49}, and that antibodies contribute to the protection induced by vaccination⁴⁸. Data from murine models also demonstrate a protective role for polymeric immunoglobulin receptor (pIgR) mediated delivery of secretory IgA, against extracellular antigens¹¹. Rather, our data reinforce the importance of eliciting robust and appropriate functional T cell responses, generating a milieu where antibody to surface exposed antigens can mediate protection. Furthermore, we conclude that high levels of anti-EB IgG in serum or secretions from uninfected women serve as a biomarker for an ineffective T cell response, identifying women at highest risk for reproductive sequelae after chlamydial infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments.

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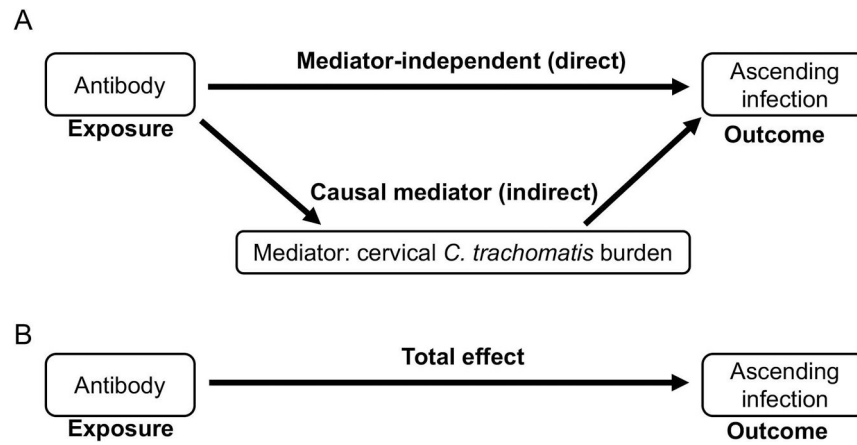


Figure 1. (A) Diagram of causal mediation analysis of the relation of antibody (Exposure), cervical *C. trachomatis* burden (Mediator) and ascending infection (Outcome). Sensitivity analysis allows for assessing unobserved confounder bias. (B) The sum of mediator-dependent and – independent effects equals the total effect.

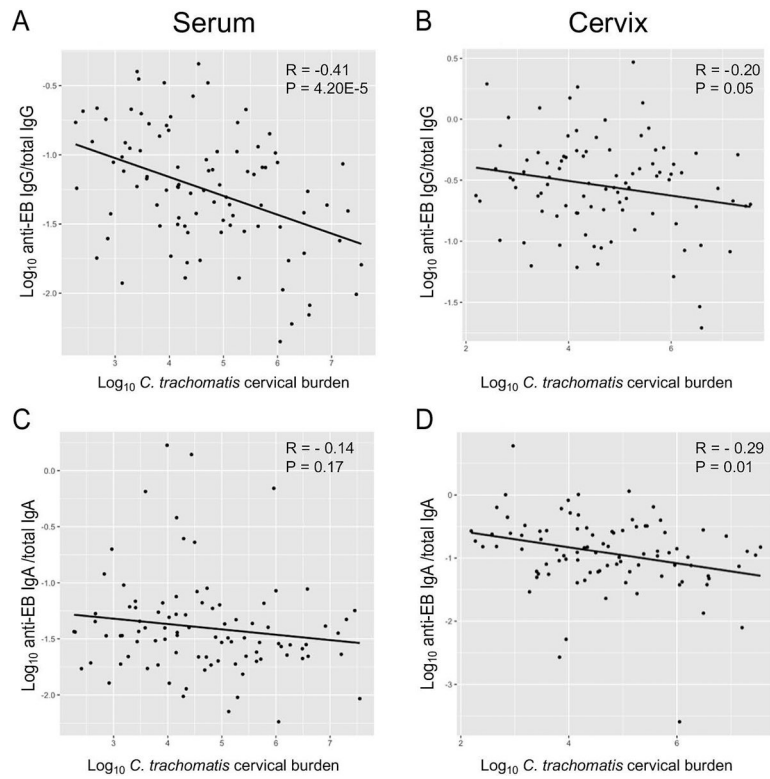


Figure 2. Correlations of cervical chlamydia burden with anti-chlamydia EB antibodies among all participants. Correlation plot for chlamydia cervical burden versus serum anti-EB IgG (A), cervical anti-EB IgG (B), serum anti-EB IgA (C), and cervical anti-EB IgA (D). X-axis: Log_{10} chlamydia DNA copies/swab. Y-axis: Log_{10} ratio of the concentration of anti-EB antibody/total antibody as determined by whole-EB ELISA.

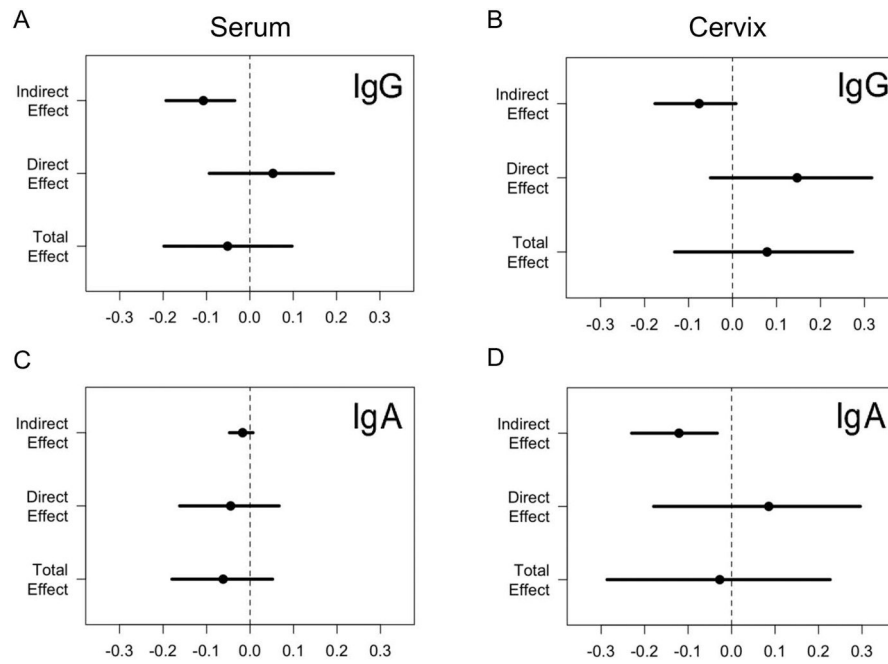


Figure 3. Graphical summary of causal mediation analysis results for effects of serum anti-EB IgG (A), cervical anti-EB IgG (B), serum anti-EB IgA (C) and cervical anti-EB IgA (D) on ascension. X-axis represents the effect value; each dot represents the point estimate, and each line represents the confidence interval. The dotted vertical line indicates absence of an effect of antibody on ascension.

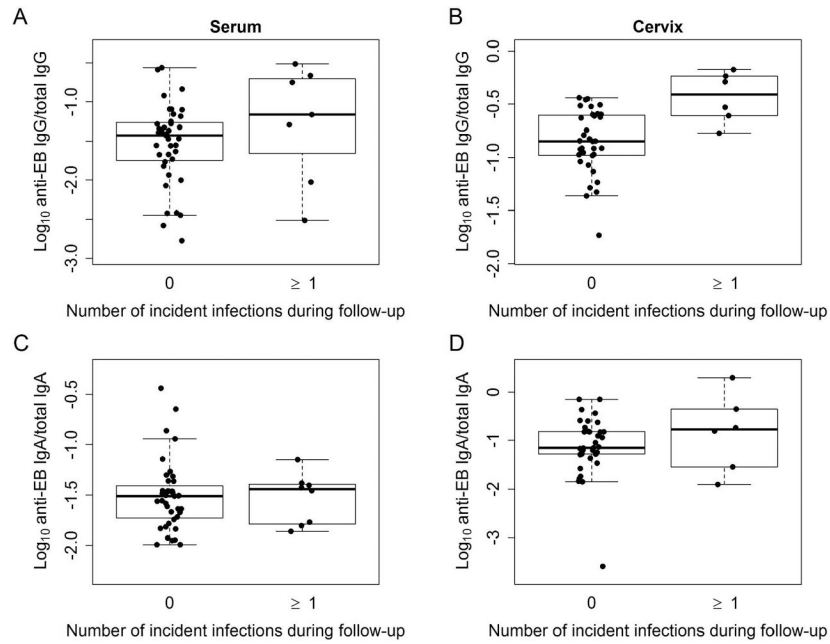


Figure 4.

Relationship of anti-EB antibodies in uninfected women at enrollment to incident infection during follow-up. Box and whisker plots for serum anti-EB IgG (A), cervical anti-EB IgG (B), serum anti-EB IgA (C), and cervical anti-EB IgA (D). Each dot represents one woman. The middle line of each box indicates the median value of antibody data. The upper and lower limit of each box represents the 75% (Q3) and 25% (Q1), respectively. The box spans the interquartile range (IQR=Q3-Q1). Upper whisker = $\min(\max(\text{antibody values}), Q3 + 1.5 * \text{IQR})$ and lower whisker = $\max(\min(\text{antibody values}), Q1 - 1.5 * \text{IQR})$.

Table 1.

Effects of antibody on probability of chlamydia ascension to the endometrium determined by causal mediator analysis.

Antibody Subtype	Average indirect effect or causal mediator effect through burden		Average direct effect independent of burden		Average total effect	
	(95% CI)	P Value	(95% CI)	P Value	(95% CI)	P Value
Serum IgG	-0.11 [#]		0.055		-0.055	
	(-0.19, -0.04)	0.001	(-0.1, 0.2)	0.445	(-0.2, 0.1)	0.496
Serum IgA	-0.02		-0.04		-0.06	
	(-0.05, 0.01)	0.180	(-0.16, 0.07)	0.452	(-0.18, 0.05)	0.296
Cervix IgG	-0.07		0.15		0.08	
	(-0.17, 0.01)	0.082	(-0.05, 0.33)	0.128	(-0.13, 0.27)	0.449
Cervix IgA	-0.12		0.09		-0.03	
	(-0.22, -0.03)	0.002	(-0.17, 0.31)	0.459	(-0.29, 0.23)	0.845

[#] Each value represents the percent decreased (-values) or increased (+values) probability of ascension. For example, there is an 11% decreased risk of ascension for women with a specific serum anti-EB IgG response.

Table 2.

Univariable analysis of baseline antibody levels \times chlamydia infection status indicates increased risk for incident infection in women who were uninfected at enrollment.

Interaction term	Description	Unadjusted		Adjusted*	
		Estimated HR [#] (95% CI)	P value**	Estimated HR (95% CI)	P value**
Serum anti-EB IgG \times chlamydia infection status	Uninfected	3.31 (1.20, 9.15)	ref***	3.63 (1.28, 10.34)	ref
	Endo-	1.04 (0.64, 1.69)	0.044	0.90 (0.51, 1.56)	0.020
	Endo+	0.85 (0.46, 1.59)	0.026	0.98 (0.51, 1.9)	0.038
Serum anti-EB IgA \times chlamydia infection status	Uninfected	0.88 (0.4, 1.93)	ref	0.89 (0.46, 1.74)	ref
	Endo-	1.23 (0.89, 1.69)	0.441	1.02 (0.72, 1.45)	0.735
	Endo+	0.77 (0.36, 1.66)	0.821	0.90 (0.42, 1.95)	0.983
Cervical anti-EB IgG \times chlamydia infection status	Uninfected	19.54 (3.17, 120.54)	ref	22.57 (3.08, 165.16)	ref
	Endo-	0.85 (0.59, 1.22)	0.001	1.05 (0.69, 1.6)	0.003
	Endo+	1.13 (0.57, 2.21)	0.004	1.27 (0.61, 2.65)	0.007
Cervical anti-EB IgA \times chlamydia infection status	Uninfected	1.16 (0.50, 2.68)	ref	1.13 (0.45, 2.85)	ref
	Endo-	1.07 (0.70, 1.65)	0.877	1.22 (0.78, 1.9)	0.890
	Endo+	0.97 (0.58, 1.63)	0.733	0.98 (0.56, 1.7)	0.790

[#]HR: hazard ratio. Each value represents the fold-change in incident infection risk for each unit increase in log₁₀ anti-EB antibody/total antibody

* Adjusted for age, gonorrhea, and sex with new, uncircumcised, or infected partners.

** P value indicates the statistical comparison of the effect of antibody on incident infection between the various groups, with uninfected women as the reference group.

ref: Reference level