SUPPLEMENT ARTICLE

Duration of Untreated, Uncomplicated *Chlamydia trachomatis* Genital Infection and Factors Associated with Chlamydia Resolution: A Review of Human Studies

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The majority of *Chlamydia trachomatis* genital infections in humans are asymptomatic and without clinical evidence of complications at the time of diagnosis. The natural history of chlamydial infection in humans, including the duration of infection and factors influencing resolution of infection, is not yet completely understood. This is in part attributable to the inherent challenges and ethical considerations in studying untreated chlamydia in humans. An improved understanding of the natural history of chlamydia in humans has implications for chlamydia screening and treatment recommendations. In April 2008, the Centers for Disease Control and Prevention convened an advisory group for the Chlamydia Immunology and Control Expert Advisory Meeting, in which studies related to chlamydia natural history, pathogenesis, and immunobiology were reviewed and gaps in our knowledge that would have implications for prevention and control of *C. trachomatis* infection were identified. This article summarizes the key questions posed and the evidence reviewed on the duration of untreated, uncomplicated genital chlamydial infection in humans and the factors associated with chlamydia resolution.

Genital *Chlamydia trachomatis* infection is the most frequently reported bacterial sexually transmitted disease in the United States, and the number of cases reported continues to increase each year. In 2007, >1.1 million chlamydial infections were reported to the Centers for Disease Control and Prevention (CDC) [1]. Increasing numbers of reported cases may simply reflect increased screening efforts rather than a true increase in disease burden, but this remains unclear. Further advances in *C. trachomatis* prevention and control efforts have been hampered in part by our limited knowledge of the natural history of untreated genital chlamydial infections in humans. Specifically, we need more information on the duration of untreated infection and the factors that influence outcomes, including spontaneous resolution (ie, chlamydia clearance without antichlamydial therapy), persistence, and the development of complications. An improved understanding of the natural history of genital chlamydial infections in humans could have implications for determining screening and rescreening intervals, timing of notification of test results and treatment, and optimizing partner notification and treatment recommendations. Differences in assumptions concerning the natural history of *C. trachomatis* infection will significantly affect estimates of screening strategy cost-effectiveness [2].

In April 2008, the CDC convened an advisory group for the Chlamydia Immunology and Control Expert Advisory Meeting, in which in vitro, animal, and humans studies related to *C. trachomatis* natural history, pathogenesis, and immunobiology were reviewed and

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knowledge gaps that would have implications for prevention and control of chlamydia were identified. This article summarizes the key questions posed and evidence reviewed on the duration of untreated, uncomplicated genital *C. trachomatis* infection in humans and factors associated with clearance. For each key question, we also address limitations of current studies and considerations in designing future studies (including ethical challenges). Animal and in vitro studies of the duration of untreated chlamydial infection that were reviewed at the advisory meeting are discussed elsewhere in this supplement [3, 4], as are human studies related to chlamydia pathogenesis and protective immunity [5–7].

PARTICIPANTS AND METHODS

Two searches of the literature were conducted on 28 January 2008 with the Medline computerized database of the US National Library of Medicine. Both searches were limited to humans, to persons ⩾13 years of age, to the English language, and to the tag terms titles or abstracts. The first search was undertaken to investigate the epidemiology of the natural history of untreated, uncomplicated chlamydial infection in humans and the factors influencing chlamydia clearance. Initially, the subject heading "chlamydia" was entered and yielded 5316 citations. These results were sequentially combined and exploded with results of searches, using the following subject headings: natural history, untreated, spontaneous resolution, spontaneous remission, duration, clearance, eradication, resolved, recurrence, relapse, remission, repeat, persistence, follow-up, and subsequent. The first search yielded a total set of 909 citations. The second search sought to investigate host and organism factors influencing susceptibility to or outcomes of uncomplicated chlamydial infection with respect to duration of untreated chlamydial infection in humans. As with the first search, the subject heading "chlamydia" was initially entered, and these results were sequentially combined and exploded with results of searches, using the following subject headings: immune, host response, genetic, microbial, biologic, and molecular. The second search yielded a total set of 418 citations.

From both sets of citations, articles were then removed if they exclusively discussed (1) *Chlamydia* species other than *C. trachomatis,* (2) complicated genital chlamydial infection (eg, pelvic inflammatory disease [PID], epididymitis, infertility), (3) chlamydia-associated reactive arthritis, or (4) in vitro research on cell lines rather than on specimens from human participants with current or prior chlamydial infection. Articles on trachoma were not excluded if they also included research on genital chlamydial infections. Citations were then reviewed individually and selected for the final set for each search on the basis of their relevance to key questions that were formulated to address the natural history of untreated, uncomplicated chlamydia in humans. Nine articles from the first search and 10 from the second were reviewed and included in this article. Two additional relevant articles during the search period that were missed by the searches were also reviewed and included, bringing the total articles included to 21 citations. In this article, the key questions and evidence reviewed are summarized in the text. Evidence tables are also included that highlight the study designs and populations, methodology, and key findings.

RESULTS

Key question 1: what is the duration of untreated, uncomplicated genital chlamydial infection in humans? Studies evaluating the natural history of untreated, uncomplicated genital chlamydial infections in humans are summarized in Table 1. The majority of chlamydia natural history studies have prospectively or retrospectively evaluated for infection resolution in the relatively short interval between initial screening and the follow-up visit for treatment or for reassessment as part of a protocol focused on another infection [8–14]. Participants in these studies did not receive specific chlamydia treatment at the time of screening because they had no indications for treatment (eg, examination findings of urethritis or cervicitis or being a contact to a sexual partner with chlamydia). Some participants received antibiotics with poor or no antichlamydial activity for other sexually transmitted diseases (STDs; eg, bacterial vaginosis, trichomoniasis, or syphilis). Follow-up intervals in most of these studies were a few weeks, although in a few instances, they were as long as several months. Chlamydia resolution rates in these studies were 11%–44%, as measured by *C. trachomatis*–specific commercial nucleic acid amplification tests or research polymerase chain reaction (PCR) assays [8–14]. Although many of these studies evaluated nonpregnant women in conventional clinical settings (eg, STD clinics, emergency rooms, and general practice clinics) [8–12], exceptions included (1) a study by Sheffield et al [13] that evaluated pregnant adolescents and adults for whom chlamydia testing was performed on archived specimens collected at baseline and follow-up visits in a multicenter bacterial vaginosis treatment trial and (2) a study by van Valkengoed et al [14] in which initial chlamydia screening was performed on a mailed urine specimen and repeat testing was performed when patients visited a general practice clinic for treatment.

Three studies evaluated the natural history of untreated, uncomplicated genital *C. trachomatis* infection in humans during intervals ⩾1 year [15–17]. In a study performed >30 years ago, McCormack et al [15] evaluated *C. trachomatis* clearance by using culture among women who were initially examined and tested for chlamydia at a university health service and then underwent reexamination and testing 15–25 months later. Of 7 chlamydia-infected women who had not received antichla-

Table 1. Studies on the Duration of Untreated Uncomplicated Genital Chlamydial Infection in Humans

Reference	Study design	Study population	Study methods	Key findings
[8]	Retrospective; 1992–1996; screening *Chlamydia trachomatis* positive and repeat *C. trachomatis* testing at treatment visit	74 participants; 93% female; most black; STD clinic; Birmingham, AL	Repeat *C. trachomatis* culture/DFA at treatment visit; *C. trachomatis* PCR on culture-negative samples	28% *C. trachomatis* resolution; resolution predicted by older age and longer interval between tests (>20 vs 4–20 days); prior *C. trachomatis* did not predict resolution
[9]	Prospective; 2002–2006; screening *C. trachomatis* positive and repeat *C. trachomatis* testing at treatment visit	129 participants; 89% female; 88% black; STD clinic; Birmingham, AL	Repeat *C. trachomatis* culture at treatment visit; *C. trachomatis* PCR on culture-negative samples; *ompA* genotyping on isolates from persisting *C. trachomatis*	18% *C. trachomatis* resolution; trend toward higher resolution with longer testing interval (median, 14 vs 12 days), male sex (36% vs 16%) and prior *C. trachomatis* infection (24% vs 13%); age did not predict resolution; participants with persisting *C. trachomatis* infection more often developed interval signs of infection
[10]	Retrospective; 1996; screening *C. trachomatis* positive and repeat *C. trachomatis* testing at treatment visit	94 participants; 62% female; one-third each black, white, and Hispanic; STD clinic; Denver, CO	Repeat *C. trachomatis* PCR at treatment visit	21% *C. trachomatis* resolution; resolution predicted by white race and ⩽3 days since last sexual activity (before initial test); resolution not predicted by testing interval, age, or prior *C. trachomatis*–associated diagnosis
[11]	Prospective; 2002–2005; screening *C. trachomatis* positive and repeat *C. trachomatis* testing at treatment visit; evaluation for *C. trachomatis* concordance within sexual partnerships	166 participants (102 partners); 62% female; 91% black; ED/GCRC; Baltimore, MD	Repeat *C. trachomatis* NAAT (LCR/PCR) and traditional *C. trachomatis* test (culture/DFA) at treatment visit; partners had *C. trachomatis* NAAT and traditional tests; 83 partnerships evaluated for *C. trachomatis* concordance	17% *C. trachomatis* resolution; resolution higher in women (23% vs 6%); no data on associations of resolution with age, testing interval, or prior *C. trachomatis* infection; higher *C. trachomatis*–positive partner concordance in NAAT positive/traditional positive vs NAAT-positive/traditional negative (75% vs 45%); partners of patients with persistent *C. trachomatis* infection more often *C. trachomatis* positive than partners of those with resolution (70% vs 11%)
[12]	Prospective; 1998; screening *C. trachomatis* positive and repeat *C. trachomatis* testing at follow-up visit	9 male participants; all Danish military recruits	Repeat *C. trachomatis* PCR at follow-up visit at 5–8 months	1 (11%) of 9 participants had *C. trachomatis* resolution; no data on associations of resolution with age, testing interval, or prior *C. trachomatis* infection
[13]	Retrospective; 1995–1998; screening *C. trachomatis* positive and repeat *C. trachomatis* testing performed	140 pregnant women; 89% black; participants from multicenter BV treatment trial; United States	Archived urine tested by *C. trachomatis* LCR at randomization (between 16 weeks 0 day and 23 weeks 6 days gestation); repeat urine LCR at follow-up visit between 24 weeks 0 day and 29 weeks 6 days gestation	44% *C. trachomatis* resolution; predictors of *C. trachomatis* resolution were older age, more lifetime partners, and longer testing interval (26% <5 weeks vs 74% ⩾5 weeks); for every 5-year increase in age, odds of positive *C. trachomatis* LCR result at follow-up decreased by 40%; no influence of BV on *C. trachomatis* resolution
[14]	Prospective; 1997–1998; screening *C. trachomatis* positive on specimen sent by mail and repeat *C. trachomatis* testing at confirmation visit	110 participants; 65% female; general population; Amsterdam	Repeat *C. trachomatis* LCR at confirmation visit	16% *C. trachomatis* resolution; no association of sex or age with resolution; no data on association of resolution with prior *C. trachomatis* infection
[15]	Prospective; 1974–1976; screening *C. trachomatis* positive by culture and repeat *C. trachomatis* testing in 15–25 months	7 female participants; university health service; Providence, RI	Repeat *C. trachomatis* culture at follow-up visit	4 (57%) of 7 with *C. trachomatis* persistence at 16–17 months after initial *C. trachomatis* testing; no data on associations of resolution with age, testing interval, or prior *C. trachomatis* infection
[16]	Retrospective; 1993–2000; screening *C. trachomatis* positive and repeat *C. trachomatis* testing performed	82 female Colombian participants from HPV natural history study; Colombia	*C. trachomatis* PCR on archived cervical scrapings collected every 6–9 months for mean follow-up of 5.7 years; *ompA* typing (nested VD2 region) on isolates (for some visits) from persons with persisting *C. trachomatis* infection	*C. trachomatis* resolution: 54% at 1 year, 82% at 2 years, 94% at 4 years; *C. trachomatis* resolution rate higher in those ever taking OCPs and with first sex at >19 years of age; *C. trachomatis* resolution not associated with age; *C. trachomatis* OmpA types from serogroups B and C lower *C. trachomatis* resolution than intermediate group; no data on association of resolution with prior *C. trachomatis* infection

Table 1. ***(Continued.)***

Reference	Study design	Study population	Study methods	Key findings
[17]	Prospective; 1995–1997; screening *C. trachomatis* positive and repeat *C. trachomatis* testing done on mailed-in specimens	30 female participants; company health services; Amsterdam	Repeat *C. trachomatis* PCR on mailed-in specimens at 1, 6, and 12 months after initial *C. trachomatis* screening; *ompA* typing (nested PCR with RFLP) on baseline isolates	*C. trachomatis* resolution rate 4.9%/month (44.7%/year); *C. trachomatis* resolution not associated with age; no data on association of resolution with prior *C. trachomatis* infection; no participants developed clinical PID by 1 year; OmpA type E infections more often persistent, but difference not statistically significant

NOTE. Only participants who did not receive antichlamydial treatment between the time of initial chlamydia screening and follow-up chlamydia testing are included in this table. BV, bacterial vaginosis; DFA, direct fluorescent antibody; ED, emergency department; GCRC, general clinical research center; HPV, human papillomavirus; LCR, ligase chain reaction; NAAT, nucleic acid amplification test; OCPs, oral contraceptive pills; *ompA*, gene encoding outer membrane protein A (OmpA); PCR, polymerase chain reaction; PID, pelvic inflammatory disease; RFLP, restriction fragment-length polymorphism; STD, sexually transmitted disease; VD, variable domain region of *C. trachomatis ompA*.

mydial therapy in the interim, 4 (57%) were infected 16–17 months after the initial testing. Molano et al [16] retrospectively evaluated *C. trachomatis* clearance with use of PCR performed on stored cervical specimens from Colombian adolescent and adult female participants who had been enrolled in a human papillomavirus natural history study and had cervical specimens collected every 6–9 months for a mean follow-up time of >5 years. Their analyses were limited to 82 female participants with normal cervical cytological findings who tested positive for *C. trachomatis* at baseline and had ⩾1 follow-up visit. Chlamydia resolution occurred in 54% of participants at 1 year of follow-up, 83% at 2 years, 91% at 3 years, and 95% at 4 years. Morré et al [17] prospectively followed 30 asymptomatic chlamydia-infected female participants for 1 year without treatment, and *C. trachomatis* nucleic acid amplification tests were performed on urine samples mailed with clinical and behavioral data questionnaires at 1, 6, and 12 months after the baseline clinic visit. The chlamydia resolution rate at 1 year was 45%, and none of the women reported clinical symptoms of PID.

A major limitation of all chlamydia natural history studies reviewed was the lack of knowledge about when participants were initially infected; this limits the accuracy of estimates for the duration of chlamydia infection. Another major limitation in most studies was not performing typing of the *C. trachomatis* outer membrane protein A (OmpA) on both the initial and subsequent chlamydia-positive specimens to ensure a more accurate classification of continued infection as either persistent (with strains of the same OmpA type) or new (with strains of different OmpA types). Geisler et al [9] previously reported that 5% of participants initially classified as having persistent chlamydial infection were subsequently found to have discordant OmpA types at the screening and treatment visits, suggesting that they had acquired a new chlamydial infection. Another limitation of most of the studies reviewed concerned the generalizability of the observations, because most studies were conducted in populations with high chlamydia prevalence rates.

In summary, human studies on the duration of untreated, uncomplicated genital *C. trachomatis* infections have shown that chlamydia clearance increases over time, with approximately half of infections spontaneously resolving ~1 year after initial chlamydia testing. However, lack of precise data on the timing of infection acquisition, lack of OmpA typing data (or other *C. trachomatis* strain typing methods), and lack of generalizable findings are major limitations of most chlamydia natural history studies to date. There have been no studies on the natural history of untreated, uncomplicated chlamydial infection in men with follow-up periods of more than a few months.

Ethical considerations are a major challenge in studying the natural history of untreated chlamydia. Untreated genital chlamydia can infrequently progress to symptomatic upper genital tract complications in men (eg, epididymitis) and women (eg, PID). There is also concern that untreated chlamydia that remains asymptomatic can still progress to cause undetected upper genital tract inflammation and damage, which has been associated with additional long-term complications (eg, ectopic pregnancy and infertility). Therefore, participants who are identified as chlamydia-infected should be treated promptly. Taking these ethical challenges into consideration, designing longitudinal studies on the natural history of chlamydia will be difficult.

Key question 2: which clinical factors influence resolution of untreated, uncomplicated genital chlamydial infection in humans? In the absence of antibiotic therapy, *C. trachomatis* clearance in humans is presumed to be mediated by host immune responses. As discussed above, one factor that has been associated with chlamydia resolution in most studies is a longer interval between the time of initial chlamydia testing and subsequent treatment, suggesting that the immune system clears the infection over time. However, it is important to understand which clinical factors are associated with a more rapid resolution of chlamydial infection, because these may be surrogates for protective immune responses. It has been postulated that prior chlamydial infections may provide protective immunity against subsequent infections. Older age, which may be a surrogate for prior chlamydial infections, was associated with chla-

mydia resolution in 2 studies [8, 13], but this association was not consistently found in all studies reviewed. Two studies [8, 9] evaluated whether a history of genital chlamydia influenced the duration of chlamydial infection. The results were contradictory; one study demonstrated a trend toward a higher frequency of chlamydia resolution among those with a history of chlamydia [9], but the other did not find a difference in resolution rates [8]. Both studies were limited, because determination of a prior chlamydia infection was based only on review of clinical and laboratory records. Because serum *C. trachomatis* antibody testing was not performed, prior chlamydia infection status may have been misclassified in patients without a reported or documented history of chlamydia. Both studies were also limited by small sample sizes, short follow-up periods, and the inability to determine precisely the duration of chlamydia infection. Moreover, 1 of the 2 studies did not incorporate *C. trachomatis ompA* typing in the classification of outcomes [8].

Two studies demonstrating an association of sex with chlamydia resolution had contradictory results; the first found a trend toward more frequent chlamydia resolution in men (36% vs 16%) [9], and the other found more frequent resolution in women (23% vs 6%) [11]. A single study reported an association between race or ethnicity and infection resolution, with infections resolved more often in white than in nonwhite participants [10]. Sheffield et al [13] evaluated whether bacterial vaginosis influences chlamydia resolution and demonstrated no association with either clinical findings of bacterial vaginosis or treatment of the condition. Finally, Molano et al [16] reported an association between oral contraceptive use and chlamydia resolution, with women who had ever used oral contraceptives clearing the infection more rapidly.

In summary, we have limited knowledge about the clinical factors that influence the duration of untreated, uncomplicated genital chlamydial infections in humans. Prior chlamydial infections may afford some degree of protective immunity and, thereby, shorten the duration of subsequent infections. Future studies addressing the influence of prior chlamydial infection on the duration of subsequent infections will need to incorporate *C. trachomatis* serological testing to define prior exposure more accurately. If possible, such studies should be designed to precisely define the timing of chlamydia acquisition and use larger samples of participants to provide sufficient power to evaluate associations of clinical factors with chlamydial infection resolution. To define more precisely the timing of chlamydia acquisition, studies would probably have to incorporate very frequent collection of genital tract samples for chlamydia testing and a diary of sexual activities in a population of participants who are negative for *C. trachomatis* at baseline but are at high risk for STD acquisition.

Key question 3: which host immune responses occur in uncomplicated genital chlamydial infections in humans, and which genetic determinants of the host modulate these immune responses? Critical to our understanding of the duration of genital chlamydial infection in humans is knowledge of the systemic (peripheral blood) and mucosal host immune responses that facilitate *C. trachomatis* clearance and the genetic determinants that modulate these immune responses. In contrast to vast amounts of animal and in vitro data on the host immune responses facilitating *C. trachomatis* clearance, there has been sparse research into the human immune responses to genital chlamydial infections, and most of the available information does not address the relationship of host immune responses to resolution of uncomplicated chlamydial infection.

Key findings from the human immune response studies reviewed (Table 2) are that, during active genital chlamydial infection, (1) serum and genital mucosal antibodies (mainly immunoglobulin [Ig] A and IgG) to specific *C. trachomatis* proteins and to *C. trachomatis* elementary bodies (EBs) are usually detected [18–21]; (2) there is an increase in genital mucosal concentrations of select cytokines [18, 21, 22]; (3) urethral polymorphonuclear (PMN) cell counts in men are highly variable, with almost 20% of men having <5 PMN cells per oil immersion field [23]; (4) PMN cell counts in cervicovaginal lavage samples are not consistently elevated [24]; (5) an increase in cervical T cell but not B cell phenotypes has been demonstrated, and there is no change in immunophenotypes systemically [25]; and (6) both systemic and mucosal lymphoproliferative responses of peripheral blood mononuclear cells to *C. trachomatis* antigens have been demonstrated [18, 20]. These studies assessed host immune responses during active chlamydial infection.

In a longitudinal study of host immune responses in commercial sex workers in Nairobi who were at high risk of chlamydia, Cohen et al [26] found that select peripheral blood mononuclear cell lymphoproliferative responses to *C. trachomatis* EBs and heat-shock protein 60 (cHSP60), measured in participants at baseline, correlated with protection against incident chlamydia, but neither cervical nor serum antibodies against *C. trachomatis* EBs and cHSP60 were associated with a decrease in incident chlamydial infection. As noted in Table 2, the study by Agrawal et al [18] also evaluated differences in host immune responses between female participants with primary genital chlamydial infection and those with recurrent genital chlamydial infections (distinguished by absence or presence, respectively, of serum *C. trachomatis* IgG). These authors found that cervical lymphoproliferative responses to cHSP10 were higher in recurrent infection, whereas lymphoproliferative responses to OmpA were higher in primary infection; another key finding was that interferon-γ levels were higher in cervical wash samples from women with recurrent infection.

A major limitation of many of the studies evaluating host immune responses in uncomplicated genital chlamydial infec-

Table 2. Studies Evaluating Host Immune Responses and Genetic Determinants in Uncomplicated Genital Chlamydial Infection in Humans

Reference	Study design	Study population	Study methods	Key findings
[18]	Prospective; host immune responses measured in *Chlamydia trachomatis*–positive patients and control subjects (no history of STDs); cases categorized as first *C. trachomatis* infection (absent serum *C. trachomatis* IgG) vs recurrent *C. trachomatis* infection	125 case patients (44 first *C. trachomatis* infection, 81 recurrent), 45 control subjects; all female; GYN department (case patients), FP clinic (control subjects); New Delhi	Cervical cells (cytobrush), cervical wash (cervical swab) samples, and blood samples collected; cervical and PBMC LP responses to cHSP10, cHSP60; and OmpA evaluated; ELISA for cervical antibody to *C. trachomatis* OmpA and cHSPs; ELISA for cervical cytokines: IL-1β, IL-6, IL-10, and IFN-γ	Cervical LP response to cHSP10 higher in recurrent *C. trachomatis* infection and to MOMP higher in first *C. trachomatis* infection; no difference in PBMC response in first vs recurrent *C. trachomatis* infection; cervical IgG/IgA to MOMP more frequent in first *C. trachomatis* infection and cervical IgG/IgA to cHSP10 and IgG to cHSP60 more frequent in recurrent *C. trachomatis* infection; cervical IFN-γ higher in recurrent *C. trachomatis* infection and correlated with cHSP60
[19]	Prospective; host immune responses measured in *C. trachomatis*–infected participants with genital symptoms and signs	50 participants (substudy: 10 female participants); 54% female; GU medicine depatment; United Kingdom	Genital swab (cervical, urethral) and blood samples collected; in substudy, women underwent cervical biopsy at baseline and 6 weeks after treatment; ELISPOT using plasmid protein pgp3 for ASCs in blood (and cervix in substudy); ELISA for serum IgA and IgG to pgp3	Blood IgA anti-pgp3 ASC response higher than IgG/IgM ASC response; serum antibodies followed same trend; cervical ASCs (mainly IgA) to pgp3 30–50 times higher in cervical samples than in blood samples and decreased 6 weeks after treatment but still present
[20]	Prospective; host immune responses measured in *C. trachomatis*–positive participants with cervicitis (cases) or asymptomatic *C. trachomatis*–negative control subjects	15 case patients, 10 control subjects; all female; GYN department; New Delhi	Cervical swab and blood samples collected; EIA for *C. trachomatis*–specific antibody in serum and cervical secretions; cervical and PBMC LP response (IL-2 activity) to PPD and OmpA L2 EBs evaluated	No difference in PBMC LP to PPD and EB in *C. trachomatis*–positive patients vs control subjects; inhibition of IL-2 production in *C. trachomatis*–positive cervical secretions from 44%–88%, but no IL-2 inhibition in control subjects; only 3 of 15 *C. trachomatis*–positive patients had *C. trachomatis*–specific cervical IgA, even though most had *C. trachomatis*–specific serum IgA; most *C. trachomatis*–positive patients had cervical and serum *C. trachomatis*–specific IgG
[21]	Retrospective; host immune responses measured in participants with NGU (*C. trachomatis* positive and *C. trachomatis* negative) or uninfected control subjects	86 patients with NGU (71 *C. trachomatis* positive, 15 *C. trachomatis* negative), 56 control subjects; all male; 70% black; STD clinic; Birmingham, AL	Urethral and serum (from a subset) specimens tested for 11 cytokines and *C. trachomatis*–specific antibodies by ELISA; urethral cells concentrated on glass slide and enumerated	Urethral IL-8 higher in *C. trachomatis*–positive patients than in *C. trachomatis*–negative persons with NGU or control subjects, but no differences in Th1/Th2 cytokines; serum IL-8 lower in *C. trachomatis*–positive patients than in control subjects, but no differences in TH1/TH2 cytokines; urethral *C. trachomatis*–specific IgA and IgG levels higher in *C. trachomatis*-positive patients than in control subjects, but no differences in serum antibody levels; lower urethral monocyte counts in *C. trachomatis*–positive patients with NGU than in control subjects; no difference in urethral lymphocyte counts between the groups; higher PMN counts in *C. trachomatis*–negative patients with NGU than in *C. trachomatis*–positive patients with NGU or control subjects
[22]	Retrospective; host immune responses measured and genetic variants determined in participants tested for *C. trachomatis* at multiple visits	396 female adolescents; 75% black; 64% HIV positive; multicenter longitudinal study (REACH); United States	Cervical *C. trachomatis* LCR and cervical lavage for IL-2 and IL-12β by ELISA every 6 months for 5 years; baseline cytokine gene typing	Cervical IL-2 lower and IL-12β higher in *C. trachomatis*-positive vs *C. trachomatis*-negative visits; these responses reversed with *C. trachomatis* clearance; no association of IL-2 and IL-12β cytokine gene variants with cytokine levels in response to *C. trachomatis* acquisition or clearance

Table 2. ***(Continued.)***

Reference	Study design	Study population	Study methods	Key findings
[23]	Retrospective; host immune responses measured in *C. trachomatis*–infected participants	2266 male participants; 91% black; STD clinic; Birmingham, AL	PMN cell counts on urethral; Gram stain quantified per oif PMN cell counts compared by *C. trachomatis* culture results (all participants were *C. trachomatis* NAAT positive)	Urethral PMNs/oif in *C. trachomatis* positive: ⩾5 in 82%, 1–4 in 6%, and 0 in 12%; *C. trachomatis*-positive participants with ⩾5 PMNs/oif more often had urethral symptoms or signs; no difference in PMN cell counts or symptoms in *C. trachomatis* culture–positive vs culture-negative participants
[24]	Retrospective; host immune responses measured in participants tested for *C. trachomatis* at multiple visits	967 female participants; majority of cohort black; 68% HIV positive; multicenter longitudinal study (HERS); United States	Cervical swab (for *C. trachomatis* EIA) and CVL samples collected every 6 months; data from 5 visits evaluated; CVL total WBC, percentage of PMN cells, and percentage of monocytes evaluated by automated cell counter	In all participants, *C. trachomatis* not associated with increased inflammatory cells in CVL; stratifying by HIV status, HIV-infected participants with *C. trachomatis* infection had higher CVL WBC counts and trend toward higher CVL PMN counts, compared with HIV-uninfected participants with *C. trachomatis* infection; progesterone-based contraception, younger age, and black race associated with increased CVL inflammatory cells
[25]	Prospective; host immune responses measured in participants with symptoms and signs of cervicitis (*C. trachomatis* positive and *C. trachomatis* negative)	18 *C. trachomatis*–positive patients, 30 *C. trachomatis*–negative participants; all female; GYN department; New Delhi	Endocervical cytobrushes/swab and blood samples collected; flow cytometry on endocervical secretions and blood samples	Increase in cervical CD3/CD4/CD8/dendritic cellular phenotypes in *C. trachomatis*–positive vs *C. trachomatis*–negative participants, but no difference in B cells; no differences in peripheral blood immunophenotypes in *C. trachomatis*–positive vs *C. trachomatis*–negative participants
[26]	Prospective; host immune responses measured in participants tested for *C. trachomatis* at multiple visits	299 female participants; 30% HIV positive; commercial sex workers; longitudinal study; Nairobi	Cervical *C. trachomatis* PCR baseline and every 2 months for 2 years; baseline cervical mucus (for antibody studies) and blood (for antibody and PBMC cytokine studies); ELISA for cervical and serum antibodies to *C. trachomatis* EBs (pooled from multiple OmpA types) and cHSP60; PBMC cytokine responses evaluated: IFN-γ and IL-5, IL-10, and IL-13	Older participants had lower incident *C. trachomatis* risk; PBMC response higher to EBs vs cHSP60 for all cytokines except IL-10; IFN-γ response to cHSP60 and IL-13 to EB correlated with protection from incident *C. trachomatis;* cervical and serum IgA/IgG levels to EBs and HSP not associated with incident *C. trachomatis* and not correlated with PBMC stimulation
[27]	Retrospective; genetic variants determined in participants tested for *C. trachomatis* at multiple visits	485 adolescents; 74% female; 70% black; 68% HIV positive; multicenter longitudinal study (REACH); United States	Genital swab *C. trachomatis* LCR at baseline and every 6 months for 5 years; HLA typing at baseline	DQB1*06 (mostly *0603 and *0603) and B*44-Cw*04 associated with incident *C. trachomatis: C. trachomatis*–positive participants more often had 1 or both variants vs *C. trachomatis*–negative participants; B*08 less often associated with incident *C. trachomatis*
[28]	Retrospective; host immune responses measured and genetic variants determined in participants tested for *C. trachomatis* at multiple visits	485 adolescents; 74% female; 70% black; 68% HIV positive; multicenter longitudinal study (REACH); United States	Genital swab *C. trachomatis* LCR and cervical lavage samples at baseline and every 6 months for 5 years; HLA and cytokine gene typing at baseline; ELISA for cervical IL-10	DRB1*03-DQB1*04 and DQB1*06 associated with recurrent *C. trachomatis* infection; a GCC haplotype defining variants at *IL10* gene promoter positions −1082/−819/−592 was underrepresented in recurrent *C. trachomatis* infection; women without the *IL10* GCC haplotype had elevated cervical IL-10 levels after *C. trachomatis* acquisition

NOTE. ASC, antigen-specific antibody secreting cell; cHSP, *C. trachomatis* heat-shock protein; CVL, cervicovaginal lavage; EBs, elementary bodies; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; ELISPOT, enzyme-linked immunosorbent spot assay; FP, family planning; GU, genitourinary; GYN, gynecology; HERS, HIV Epidemiology and Research Study; HIV, human immunodeficiency virus; IFN, interferon; IgA and IgG, immunoglobulin A and G; IL, interleukin; LCR, ligase chain reaction; LP, lymphoproliferative; NAAT, nucleic acid amplification test; NGU, nongonococcal urethritis; oif, oil field on light microscopy (×1000); OmpA, *C. trachomatis* outer membrane protein A; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; PMN, polymorphonuclear leukocyte; PPD, purified protein derivative; REACH, Reaching for Excellence in Adolescent Care and Health Study; STD, sexually transmitted disease; Th1 and Th2, T-helper 1 and 2; WBC, white blood cell.

tions in humans was the lack of control for other potential pathogens (eg, herpes simplex virus, *Mycoplasma,* and *Ureaplasma*) that could induce immune responses (eg, cytokines and cellular phenotypes) that are not specific for *C. trachomatis.* Another limitation in some studies was lack of generalizability of the observations beyond the patient populations studied, such as studies involving human immunodeficiency virus (HIV)–infected participants or participants with multiple prior STDs.

Understanding host immune responses in uncomplicated genital chlamydial infections in humans is just as important as understanding the host genetic determinants that modulate these immune responses. It is often difficult to link specific genetic determinants to specific host immune responses, and the 2 studies that evaluated the relationship of genetic determinants and uncomplicated genital chlamydial infections have primarily focused on how the genetic determinant affected risk of chlamydia acquisition [27] or recurrence [28], rather than on host immune responses to the infection (Table 2).

These 2 studies, by Geisler et al [27] and Wang et al [28], evaluated data from 485 adolescents who were enrolled in the Reaching for Excellence in Adolescent Care and Health (REACH) study, a multicenter, longitudinal study of adolescent health. Genetic variants determined at baseline included HLA class I and II variants and 16 single-nucleotide polymorphisms from 7 cytokine genes. *C. trachomatis* ligase chain reaction testing was performed every ~6 months for a median follow-up period of 3 years. The majority of participants were female, black, and HIV infected. Geisler et al [27] found that DQB1*06 and/or B*44-Cw*04 predicted incident chlamydial infection (defined as a new chlamydial infection during follow-up in participants negative for chlamydia at baseline) independent of prior chlamydial infection. Wang et al [28] found that an interleukin-10 gene promoter variant (promoter positions −1082, −819, and −592) was protective against recurrent chlamydia (defined as ⩾2 chlamydial infections during the study separated by a visit during which the patient tested negative for chlamydia), and female participants without this genetic variant had higher endocervical interleukin-10 levels at the time of chlamydia acquisition. The major limitations of these studies were the inclusion of a significant number of HIV-infected participants, which could have confounded immune responses, and the fact that the populations were primarily black, which limited the generalizability to persons with other races, especially with regard to differences in genetic backgrounds. Another limitation of the REACH cohort studies was analysis of a small number of genetic variants.

In summary, there are few studies of host immune responses in humans with uncomplicated genital chlamydial infections. These studies evaluated immune responses during active chlamydial infection but did not evaluate which specific host responses affect infection resolution versus persistence. There are also few studies on the role of immunogenetic determinants of the outcome of uncomplicated genital chlamydial infection (in contrast, trachoma or tubal factor infertility have been studied more extensively). Future studies of the relationship of host immune responses and genetic determinants with uncomplicated chlamydial infection resolution will need to address the limitations of studies to date. Such future studies will need (1) a more exhaustive evaluation for other potential pathogens that could influence immune responses, (2) a healthy, diverse patient population, (3) more detailed evaluations of host immune responses against a wider range of *C. trachomatis* antigens, and (4) use of high-throughput DNA sequencing technology to screen a much larger number of genetic determinants (not only HLA and cytokine gene variants but also, perhaps, repetitive DNA elements).

Key question 4: which biological properties of* C. trachomatis *influence resolution of uncomplicated genital chlamydial infections in humans? Although there have been considerable advancements in understanding of the biological and functional characteristics of *C. trachomatis,* there has been sparse translational research evaluating how the biological characteristics of *C. trachomatis* impact uncomplicated genital chlamydial infection outcomes in humans. The primary biological characteristic of *C. trachomatis* of which the relationship to resolution of uncomplicated genital chlamydial infection in humans has been studied is the OmpA protein (previously referred to as major outer membrane protein, or omp1). OmpA is an abundant *C. trachomatis* protein that is exposed on the surface of the organism and is highly immunogenic. Three studies used *ompA* genotyping (by different methods) to evaluate the relationship of *C. trachomatis* OmpA genotype with uncomplicated genital chlamydial infection resolution or persistence in women [16, 17, 29]. Molano et al [16] demonstrated that female participants infected with *C. trachomatis* serogroup B and C OmpA types had a longer duration of genital chlamydial infection (ie, persistence). Morré et al [17] reported that female participants infected with *C. trachomatis* OmpA type E were more likely to have chlamydia persistence after 1 year of follow-up than were those infected with the other genotypes. Both of these studies were limited, because OmpA genotyping was not performed at all visits and men were not evaluated.

Dean et al [29] described several female participants with ⩾3 consecutive, uncomplicated genital chlamydial infections with the same OmpA genotype (identical *ompA* gene sequences or only 1 or 2 amino acid changes) during >2 years of follow-up at a Seattle STD clinic and suggested that this was evidence for persistence, although reinfection from an untreated partner could not be excluded. The minimum inhibitory concentrations of doxycycline and azithromycin for the *C. trachomatis* strains isolated from these participants were not elevated, ar-

guing against antimicrobial resistance contributing to persistence.

A study by Gomes et al [30] evaluated the relationship of OmpA genotype with another biological characteristic of *C. trachomatis* infection, the infectious load (a quantitative measure of organism burden and presumably a surrogate for chlamydia replication), and how genotype correlated with genital chlamydial infection outcomes. Chlamydia load was based on the copy number of organisms per copy number of eukaryotic cells, both measured by real-time quantitative PCR. Rather than study the relationship of OmpA genotype and chlamydia load to chlamydial infection resolution over time, these authors studied differences in the chlamydia load in those with prior chlamydia (controlling for OmpA genotype) and changes in the chlamydia load with subsequent chlamydia infections. Their study demonstrated that chlamydial infectious burden was initially not associated with OmpA genotype but was lower in subsequent chlamydia episodes, regardless of genotype, among in patients with prior infections, although this difference did not reach statistical significance. Neither clinical signs nor symptoms of infection were associated with organism load, and the proportion of patients in this study who had clinical findings of PID is unclear.

In summary, we have very limited knowledge of the impact of biological characteristics of *C. trachomatis* on outcomes of uncomplicated genital chlamydial infection in humans, including most importantly, the duration of infection. From the studies reviewed, it is reasonable to speculate that humans do not develop complete protective immunity to a specific OmpA genotype but that they develop partial protective immunity, as evidenced by the lower organism loads with subsequent genital chlamydial infection. Since the sequencing of the *C. trachomatis* genome, there has been an acceleration in the in vitro work describing biological characteristics and possible life cycle variants of *C. trachomatis*. Future studies must translate these findings into human data to improve understanding of the clinical implications in the natural host of this important sexually transmitted pathogen (eg, which *C. trachomatis* proteins stimulate immune responses in humans, resulting in more rapid chlamydia resolution and/or protection against recurrent infection).

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