

Immunoglobulin-Based Investigation of Spontaneous Resolution of *Chlamydia trachomatis* Infection

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FOOTNOTES

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ABSTRACT

Chlamydia trachomatis (CT) elementary body (EB) ELISA was used to investigate serum anti-CT IgG1 (long-lived response) and IgG3 (short-lived response indicating more recent infection) from treatment (enrollment) and 6-month follow-up visits in 77 women previously classified as having spontaneous resolution of chlamydia. 71.4% of women were IgG1+IgG3+, consistent with more recent chlamydia resolution. 15.6% were IgG3- at both visits, suggesting absence of recent chlamydia. Using EB ELISA, we demonstrated about one in six women classified as having spontaneous resolution of chlamydia might have been exposed to CT but not infected. Further, we classified their possible infection stage.

Key words:

chlamydia, immunoglobulin, antibody, IgG1, IgG3, resolution, clearance, EB ELISA

BACKGROUND

Persisting genital *Chlamydia trachomatis* (CT) infection can lead to chronic inflammation, which may result in pelvic inflammatory disease and tubal factor infertility. Studying the natural clearance of CT infection in humans is ethically challenging because CT detection obligates treatment to eradicate infection and limit complications. Sparse studies that tested stored specimens suggest that ~50% of CT infections spontaneously resolve (without treatment) within one year, presumably through immune-mediated clearance [1, 2]. Most studies on spontaneous resolution of CT infection have reported the resolution frequency between the time of CT screening (typically with a nucleic acid amplification test [NAAT]) and returning for follow-up, usually for treatment of a positive test. Those with a positive CT screening test but negative CT test at follow-up are classified as having spontaneous resolution, which studies report occurs in 11% to 44% of individuals within a few weeks to several months of a positive screening CT NAAT [3]. We found a spontaneous resolution frequency of ~20% in subjects studied at a STD Clinic in Birmingham, AL [4].

The potential clinical significance of spontaneous resolution is that patients who clear CT infection before treatment have a lower reinfection risk than those with persisting infection [5]. It has been suggested this may be because patients whose infections spontaneously resolve develop protective immunity, in contrast to those with persisting infection having “arrested” immunity if treated too early in their infection [6]. However, some patients classified as spontaneous resolution based on a repeat NAAT being negative may have been misclassified. NAAT

detects nucleic acids and cannot distinguish viable from nonviable organisms. Thus, NAAT cannot differentiate established infection from exposure (i.e., from CT in a partner's secretions) that does not lead to infection. In contrast, culture only detects viable organisms and was used to define spontaneous resolution in sparse studies [7, 8], but is less sensitive than NAAT and not widely available. Neither NAAT nor culture, if positive, provides information on potential timing/duration of the recently resolved CT infection; some infections could be "acute" (early stage of infection) or "primary" (an individual's first CT infection). Neither test can identify a "remote" infection (in the distant past; likely years ago).

We previously used a CT elementary body (EB) ELISA to characterize CT-specific immunoglobulin (Ig) responses in individuals with a positive CT NAAT and found that IgG1 and IgG3 comprised the predominant serum anti-CT Ig response [9]. Among IgG subclasses, IgG1 has the longest half-life and is most abundant [10], whereas IgG3 is of lower abundance with a shorter half-life. IgG3, however, is the first IgG subclass to increase following infection and is associated with effector functions, including antibody dependent cell-mediated cytotoxicity and neutralization [10]. In this study, we used EB ELISA to measure anti-CT IgG1 and IgG3 responses in women previously classified as having spontaneous resolution of CT infection to address the following objectives: 1) distinguish true spontaneous resolution vs. CT exposure without established infection, and 2) delineate differences in timing/duration of CT infection by measuring differences in duration of anti-CT IgG1 and IgG3 responses [10], including measurements at a 6-month follow-up visit to assess for

seroconversion after treatment.

METHODS

Study Participants and Clinical Procedures

Our study evaluated sera and clinical data previously collected from women returning to the Jefferson County Department of Health (JCDH) STD clinic in Birmingham, AL for treatment of a recent positive screening CT NAAT who were enrolled into a CT natural history study. Investigations focus on women classified as having spontaneous resolution of CT infection based on a negative repeat CT NAAT at enrollment, at which time women were interviewed regarding their medical and sexual history, underwent phlebotomy, had a cervical swab collected for repeat CT NAAT (Hologic Aptima Combo 2 [AC2]; Hologic, Inc., Marlborough, MA), and were treated with azithromycin 1g. Participants had a 6-month follow-up visit scheduled. Written informed consent was obtained from patients before enrollment. The study was approved by the University of Alabama at Birmingham Institutional Review Board (IRB) and JCDH. The Centers for Disease Control and Prevention (CDC) determined that CDC involvement did not constitute engagement in human subjects research, and CDC IRB review was therefore not required.

CT EB-ELISA

CT-specific IgG1 and IgG3 responses were measured by EB ELISA as described previously [9, 11, 12]. Briefly, ELISA was performed using formalin-fixed CT EBs

pooled from serovars D, F and J. IgG1 and IgG3 responses were detected using alkaline phosphatase-labeled mouse anti-human IgG1 (a pool of clones 4E3, Southern Biotech; and HP6069; Cal Biochem) and mouse anti-human IgG3 (clone HP6050; Southern Biotech) at an optical density of 405 nm (OD₄₀₅). The cutoff OD₄₀₅ values for positive IgG1 and IgG3 anti-CT responses were ≥ 0.35 and ≥ 0.1 , respectively. Each subject's serum was run in triplicates at a 1:32 dilution.

Statistical Analysis

Differences in EB ELISA IgG1 and IgG3 OD₄₀₅ readings from treatment (i.e., enrollment) to follow-up visits for women categorized into different CT infection groups (described in the results below) were analyzed with SAS 9.3 (SAS Institute, Cary, NC) using the Wilcoxon signed rank test. Differences in OD₄₀₅ readings between groups were analyzed using the Wilcoxon rank sum test. Only groups with ≥ 5 women were analyzed.

RESULTS

Frequency of IgG1 and IgG3 Seropositivity in Women Identified as having Spontaneous Resolution of CT Infection

Of 108 women identified with spontaneous resolution of CT infection, 77 had serum for serological testing from both treatment and follow-up visits. The median age was 24 years (range 16-41) and 94.0% were non-Hispanic African Americans. The median interval between CT screening and treatment visits was

13 days (range 4-45) and between treatment and follow-up visits was 184 days (range 38-315). At the treatment visit, 74 (96.1%) women were seropositive for IgG1, 65 (84.4%) for IgG3, and 74 (96.1%) for IgG1 and/or IgG3. At the follow-up visit, 71 (92.2%) subjects were seropositive for IgG1, 61 (79.2%) for IgG3, and 73 (94.8%) for IgG1 and/or IgG3.

Using Anti-CT IgG3 Seropositivity Status to More Accurately Classify Spontaneous Resolution of CT infection

IgG3 is the first IgG subclass to increase upon infection [10], therefore, it would be expected that most CT-infected individuals would be IgG3 seropositive. However, a small proportion of women could be initially IgG3 seronegative if the infection was acute and, in that instance, they would be expected to seroconvert within a few weeks. Of 77 subjects classified as having spontaneous resolution, 15 (19.5%) were IgG3 seronegative at the treatment visit; 3 seroconverted IgG3 at follow-up while the other 12 remained IgG3 seronegative. Thus, the positive CT NAAT at the screening visit in 12 (15.6%) subjects classified as having spontaneous resolution of CT infection may reflect an exposure to CT without an established CT infection.

Evaluating Combinations of Anti-CT IgG1 and IgG3 Serostatus to Predict Timing/Duration of CT infection

As previously discussed, IgG1 and IgG3 differ in response time and half-life [10], thus we assessed whether different IgG1 and IgG3 seropositive/seronegative

combinations from both visits could be used to predict timing/duration of CT infection. We created the following categories (participant groups) of timing/duration of CT infection based on expected differences in IgG1/IgG3 serostatus: *recent infection* (IgG1+ and IgG3+ at the treatment visit; IgG3+ at the follow-up visit), *primary infection* (IgG1- at the treatment visit; seroconversion to IgG1+ at follow-up); *acute infection* (IgG3- at the treatment visit; seroconversion to IgG3+ at follow-up); *remote infection* (IgG1+ at the treatment visit; IgG3- at follow-up); and *no infection* (IgG1- and IgG3- at both treatment and follow-up visits). Table 1 summarizes results from our cohort. The majority, 57/77 (74.0%), were categorized as resolution of recent infection. 2 (2.6%) were categorized as resolving primary CT infection and 2 (2.6%) with resolution of acute infection. 13 (16.9%) were categorized as having remote infection, meaning they likely had CT infection years ago but not recently. There were 3 women with no serological evidence of infection, suggesting despite CT antigen exposure (positive screening CT NAAT), they have never acquired CT infection. Our data highlight the heterogeneity in IgG1 and IgG3 responses that exists among women classified as having spontaneously resolution of CT infection.

Effect of Predicted Timing/Duration of CT infection on Changes in IgG1 and IgG3 Responses between Treatment and Follow-up Visits.

Illustrated in Figure 1, we analyzed the change in median OD₄₀₅ IgG1 and IgG3 anti-CT responses between treatment and follow-up visits to determine if the magnitude of the responses correlated with the categories of CT infection shown

in Table 1. Women resolving recent infection had a significant decrease in the OD₄₀₅ for IgG3 from the treatment to follow-up visit ($P = .010$), but no significant change in OD₄₀₅ for IgG1. The small number of women that resolved primary or acute CT infection had an increase in OD₄₀₅ for both IgG1 and IgG3 at the follow-up visit. The remote CT infection group had no significant change in the OD₄₀₅ for both IgG1 and IgG3 responses between visits, and the CT-exposed women who never had infection also showed no noticeable change in the OD₄₀₅ for IgG1 and IgG3 between visits. The recent infection group had a significantly higher OD₄₀₅ for IgG1 and IgG3 compared to those with remote infection ($P < 0.001$ for both IgG1 and IgG3), possibly reflecting boosted antibody responses from recent CT exposure. Overall, changes in the median OD₄₀₅ for IgG1 and IgG3 responses were consistent with those expected for the defined categories.

DISCUSSION

Our investigation of CT-specific IgG1 and IgG3 responses in women classified as having spontaneous resolution of CT infection contributed two main findings that advance our understanding of the natural history of CT infection: 1) some women classified as having spontaneous resolution based on CT NAAT results may have been misclassified and actually were exposed to CT but never infected; and 2) among women who spontaneously resolved infection, there exists heterogeneity with respect to infection timing/duration.

Persisting CT infection generally elicits CT-specific antibody responses. Failure to detect anti-CT antibody responses after sufficient follow-up in those

with a positive genital CT NAAT suggests either they lack the ability to generate antibody responses or were not infected, rather were only exposed. We found 12/77 (15.6%) of women classified as spontaneous resolution never elicited an IgG3 response at treatment and follow-up visits: 9 of 12 were IgG1 positive, likely reflecting a remote CT infection and confirming their ability to mount anti-CT antibodies. However, 3 of 12 were never seropositive for either IgG1 or IgG3 at the treatment or follow-up visits, most likely because they were never infected and their positive screening CT NAAT may have reflected a CT exposure, although we cannot rule out infection with absence of antibody responses. The minimal change in IgG1 and IgG3 OD₄₀₅ readings between treatment and follow-up visits in these 12 women classified as having spontaneous resolution further supports absence of CT infection. This suggests one in six women were misclassified as having spontaneously resolved a CT infection. Women categorized in the recent infection group showed no change in IgG1 and a decline in IgG3 (Figure 1), which is in line with our published findings showing IgG3 responses, with a shorter half-life than IgG1, start to decline within 6 months after infection eradication [9]. In contrast, women categorized with either primary or acute infection showed an increase in IgG1 and IgG3, reflecting detection of their infection at an earlier stage. With the lack of behavioral data in our study, we cannot exclude the possibility that subjects had re-exposures to CT that could have affected magnitude of antibody responses at follow-up.

Overall, our findings demonstrate the importance of including anti-CT immunoglobulin measures with CT NAAT results for a more accurate study

classification of spontaneous resolution of CT infection. Our findings will pave the path for future studies related to the longevity of CT-specific immune responses in humans, which were previously not possible because of lack of sufficient knowledge about timing/duration of CT infection. Delineating primary vs. acute infection and remote vs. recent infection will be important in future studies investigating immune responses in individuals classified as having spontaneous resolution because presence vs. absence of recent infection and timing/duration of infection could affect immune response measures.

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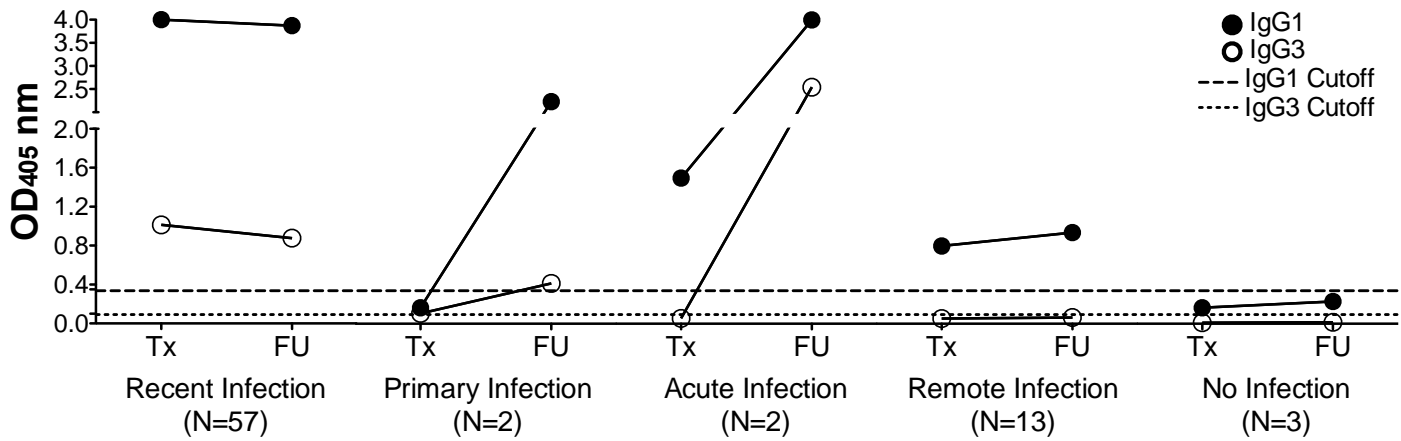


Figure 1. Effect of timing/duration of *Chlamydia trachomatis* (CT) infection on the change in the magnitude of anti-CT IgG1 and IgG3 responses between treatment and follow-up visits. Line graph depicting the changes in the median OD₄₀₅ of anti-CT IgG1 and IgG3 responses detected by CT EB ELISA from a CT treatment visit (Tx) to a follow-up (FU) visit (on average ~6 months after treatment). Closed and open circles represent median OD₄₀₅ value for IgG1 and IgG3 responses, respectively. The different infection timing/duration categories that were defined based upon seropositivity of IgG1 and IgG3 at Tx and FU visits are indicated on the x-axis. Dashed and dotted lines indicate the EB ELISA OD₄₀₅ cutoff for IgG1 (0.35) and IgG3 (0.1), respectively. In the recent infection group, the magnitude of the IgG3 response significantly declined between the Tx and FU visits (median OD₄₀₅ 1.012 vs. 0.876; $P = 0.010$), however there was no significant change in the IgG1 response median OD₄₀₅ 4.000 vs. 3.872 ($P = 0.442$). In the remote infection group, there was no significant change in the magnitude of the IgG1 or IgG3 response between Tx and FU visits (IgG1: median OD₄₀₅ 0.798 vs. 0.936

[$P = 0.735$]; IgG3: median OD_{405} 0.053 vs. 0.063 [$P = 0.733$]). The magnitude of the IgG1 and IgG3 responses were significantly higher at both the Tx and FU visits for the recent infection group vs. the remote infection group (all P values <0.001). Due to the small sample size in the acute, primary, and no infection groups ($N < 5$ in each group), statistical analyses were only performed for the recent and remote infection groups.

Table 1. Classification of *Chlamydia trachomatis* (CT) infection based on different combinations of anti-CT IgG1 and IgG3 serostatus from women classified as having spontaneous resolution by NAAT.

Type of Infection	IgG subclass responses				# Subjects (%)
	Treatment visit		Follow-up visit		
Recent^a	IgG1+	IgG3+	IgG1-	IgG3+	2 (2.60)
	IgG1+	IgG3+	IgG1+	IgG3+	55 (71.40)
Primary^b	IgG1-	IgG3+	IgG1+	IgG3+	1 (1.30)
	IgG1-	IgG3-	IgG1+	IgG3+	1 (1.30)
Acute^c	IgG1+	IgG3-	IgG1+	IgG3+	2 (2.60)
Remote^d	IgG1+	IgG3+	IgG1-	IgG3-	1 (1.30)
	IgG1+	IgG3+	IgG1+	IgG3-	3 (3.90)
	IgG1+	IgG3-	IgG1+	IgG3-	9 (11.70)
No Infection	IgG1-	IgG3-	IgG1-	IgG3-	3 (3.90)

^a Recently resolved CT infection.

^b An individual's first CT infection

^c Early stage of infection

^d Individual was infected in the distant past (likely years ago)