Chlamydia trachomatis—*The Persistent Pathogen*

Thomas Parran Award Lecture

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LET ME BEGIN by expressing my deepest gratitude to the American Sexually Transmitted Diseases Association (ASTDA) for selecting me to receive this very prestigious award. Certainly, recognition of accomplishment from one's colleagues is the most meaningful form of professional praise. Whatever my accomplishments have been, they reflect the superb training, mentorship, and guidance I've benefited from throughout my career. My interest in epidemiology, public health, and research was kindled by Dr. John V. Bennett when I worked at the Centers for Disease Control and Prevention (CDC). Upon returning to Seattle for an Infectious Diseases fellowship, I was fortunate to be recruited into sexually transmitted disease (STD) research by King K. Holmes. He then provided unparalleled mentorship in virtually all aspects of a successful academic career. The opportunities, advice, teaching, and inspiration that he provided, not just during my fellowship but also throughout my career cannot be overstated, and I am greatly appreciative.

I've also had the pleasure and opportunity to work with a host of other very talented colleagues. While time does not permit a complete listing, I'd particularly like to mention fellow University of Washington faculty members Larry Corey, Hunter Handsfield, and Mac Hooton, as well as David Eschenbach, Dorothy Patton, Mary Lampe, and Delia Scholes; in addition, former Seattle fellows or faculty, including David Martin, Robert Jones, Thomas Quinn, Jorma Paavonen, Robert Brunham, Ned Hook, Ann Rompalo, and Kim Workowski; and finally, the many colleagues I have worked with in the chlamydia field, especially Julius Schachter and Russell Alexander, who first taught me about this peculiar organism, and the staff of the Chlamydia Research Laboratory at the University of Washington.

Supported in part by grants and contracts from the National Institutes of Health (AI-31448, AI-48769, N01-AI-75329).

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Received for publication August 6, 2001, and accepted August 10, 2001.

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A lifetime achievement award like this one provokes self-reflection and a retrospective view of one's career. In thinking back over both my own studies with chlamydia and our concepts of chlamydial epidemiology, pathogenesis, and approaches to prevention over the last 25 years, I am struck by the remarkable extent to which our thinking in all of these areas has been shaped by the laboratory tests available to us within each time period. It is from this perspective that I would like to review the evolution of the field this morning.

The Chlamydia Cell Culture Era (1975–1985)

Following its initial description by Gordon and Quan,[1] chlamydia cell culture became widely available to researchers and public health programs in the 1970s. The decade from 1975 to 1985 can be considered the chlamydia culture era. A major consequence of the availability of chlamydia cultures was the ability to undertake studies to link *Chlamydia trachomatis* to specific clinical syndromes (Table 1). With regard to the urogenital tract, chlamydia was initially linked to nongonococcal urethritis, to mucopurulent cervicitis, and subsequently to pelvic inflammatory disease (PID) and a host of other complications and less frequent sites of urogenital infection.[2] My own initial research in chlamydiology focused on linking chlamydial infection to the acute urethral syndrome in women. We demonstrated that chlamydia-associated pyuria was frequent among young women with acute dysuria and urine cultures negative for bacteria. Further, these women responded to specific therapy for chlamydial infection.[3,4] From a prevention perspective, the culture era focused upon teaching clinicians to recognize these chlamydia-associated syndromes and provide empiric treatment without actual diagnostic testing of patients with these syndromes or their partners.[5] In this era, it was also

TABLE 1. Culture Era, 1975–1985

- Linking *Chlamydia trachomatis* to clinical syndromes
- Teaching recognition of these syndromes
- Empiric treatment without testing
- Empiric treatment of partners
- Treat gonorrhea with *C. trachomatis* active drugs
- Focus on sexually transmitted disease/family planning clinics
- Test asymptomatic women if feasible

demonstrated that chlamydia was frequently associated with gonococcal infection. Therefore, empiric treatment of gonorrhea with chlamydia-active drugs became an additional addendum to efforts to control chlamydial infections and was shown to reduce the occurrence of postgonococcal urethritis, cervicitis, and PID.[6] The focus of these prevention efforts was largely directed at patients attending STD and family planning clinics. Due to its expense and technical difficulty, chlamydia testing by cell culture never became widely available, however, and thus screening programs were not feasible. But where cultures were available, they were directed at the detection of unrecognized infection in asymptomatic women.

Nonculture Tests for Chlamydia (1985–1995)

The development in the mid-1980s of monoclonal antibodies for chlamydia resulted in the first nonculture test for this organism, namely, the direct fluorescent antibody (DFA) test in which fluorescein-labeled monoclonal antibodies were used to identify chlamydia elementary particles in genital secretions. The development of the DFA was followed shortly by the development of enzyme immunoassays (EIAs)for chlamydia. The nonculture era, initially utilizing DFA and EIA testing, allowed, for the first time, widespread access to clinic-based testing for chlamydia.[7] The availability of these tests led to increased opportunities for screening adolescent women and other high-risk groups (Table 2). The concept of developing selective screening criteria to facilitate more cost-effective screening of young women was explored and implemented.[8] It was also advocated that testing be undertaken to confirm the diagnosis in women with suspected mucopurulent cervicitis and PID, and to specifically test their sexual partners where possible as well.[9] In addition, screening pregnant women and selective screening in low prevalence populations became feasible for the first time. Although expanded use of these new diagnostic tests made it possible to specifically treat chlamydial infection in some individuals, most treatment remained syndromic and empiric.

TABLE 2. DFA/EIA Nonculture Era, 1986–1994

- Increased access to clinic-based testing
- Focus on screening adolescent women
- Test to confirm mucopurulent cervicitis/pelvic inflammatory diseases; test sex partners
- Screen pregnant women
- Selective screening in low prevalence populations
- Continue strategies for antibiotic treatment

DFA, direct fluorescent antibody; EIA, enzyme immunoassay.

TABLE 3. Characteristics and Implications of NAATS Testing

- Substantially improved sensitivity of testing
- Novel specimen types: urine, tampons, vaginal swabs
- Multipathogen testing
- Access to new populations
- Outreach beyond clinics:
  - Women: no pelvic exam
  - Men: No swabs
- Reduced clinician need

NAATS, nucleic acid amplification.

The Nucleic Acid Amplification Test Era (1990–Present)

In the early 1990s, the nucleic acid amplification (NAATS) tests became available to researchers, and in the mid 1990s they became available for routine clinical use and for use in public health prevention programs. These tests have had a major impact on both our understanding of the epidemiology of chlamydial infections and approaches to prevention and control of *C trachomatis* urogenital infections (Table 3). The three unique characteristics of these tests that have been so important include: (1) their improved sensitivity; (2) the ability to utilize novel specimen types, such as first-void urine, patient collected tampons, or vaginal swabs; and (3) the ability to test for multiple pathogens simultaneously.[10] The three tests that were first introduced (the ligase chain reaction [LCR], polymerase chain reaction [PCR], and transcription mediated amplification [TMA] tests) all have enhanced the sensitivity of detecting urogenital chlamydial infection by approximately 20% beyond that of former tests. In particular, these tests allow detection of infections characterized by very low numbers of inclusion-forming units (IFUs).

Among the important implications of these test characteristics has been access to new patient populations that could not be readily tested with previous modalities. The facilitation of outreach programs, i.e., testing in settings where women are not undergoing pelvic examinations and where men are not having urethral swabs collected, has been an extremely important expansion of chlamydial control efforts. In addition, NAATS testing allows collection of specimens in settings where a physician is not present.

NAATS tests have now been widely used in research and in control programs for approximately 6 years. This cumulative experience has resulted in considerable revision of our views of the clinical epidemiology of *C trachomatis* urogenital infections and consequent alterations in programs

TABLE 4. Revision of CT Epidemiology in the NAATS Era, 1995–Present

- Increased prevalence in most populations
- Increased recognition of and emphasis on asymptomatic infections
- Increased prevalence in male sex partner
- Screen all clinic attendees
- Community-based outreach screening
- Increased appreciation of recurrence/persistence
- Screen asymptomatic men; rescreen women

CT, *Chlamydia trachomatis.*

for their control. These revisions are summarized in Table 4. First, because of their increased sensitivity and ability to detect low inclusion count infections, the NAATS tests have demonstrated an increased prevalence of infection in virtually every population tested as compared with that previously described. Many of these newly recognized infections have been asymptomatic, and there has been a corresponding increased emphasis on such asymptomatic infections. For example, Gaydos and colleagues[11] used LCR urine testing to screen 13,204 female recruits entering the US Army. The overall prevalence of chlamydial infection in these recruits was 9.2% and was even higher (12.2%) among 17 year olds. The majority of the infections were asymptomatic. Mertz and colleagues[12] reported a preliminary evaluation of the NHANES III data in which persons in randomly selected households were surveyed using urine PCR. The prevalence of infection was 2% to 7% and was higher in adolescent women; again, almost all infections were asymptomatic. In another study, Klausner and colleagues[13] reported an interesting door-to-door household cluster survey evaluating 1439 women 18–29 years of age by urine LCR. They demonstrated a 3.2% prevalence of infection overall that was even higher in adolescent women.

Another concept that has changed as a result of NAATS testing has been that female partners of men infected with chlamydia test positive for chlamydia more frequently than the reverse, suggesting that chlamydial infection may be more transmissible in the male-to-female direction. In fact, when couples are tested by PCR rather than culture (facilitating the identification of low inclusion count infections), partners of both infected men and infected women are shown to have an approximately equal prevalence of infection.[14]

In terms of prevention, the NAATS era has led to expanded efforts to screen asymptomatic young women using self-collected vaginal swabs or first-void urines. New venues have been undertaken for identifying asymptomatically infected adolescents, a group that may be least likely to be encountered in routine clinical care. These approaches include street-based testing, high school–based testing, and testing military recruits. Cohen and colleagues,[15] for example, demonstrated the potential effectiveness of school-based screening for chlamydia using LCR testing. They were able to access 32% to 65% of students each year in three public high schools in the New Orleans area. They identified 11% of girls and 6% of boys as infected with chlamydia. They also showed that the peak range for chlamydia infection in boys was older than that in girls, being highest among twelfth-grade boys, and highest among ninth-grade girls. Ninety percent of the infections they identified were asymptomatic. Importantly, they demonstrated that upon repeated testing, the chlamydial prevalence in boys decreased as compared with boys at control schools who did not have ongoing testing. Another interesting example of outreach testing has been that pioneered by Ostergaarde and colleagues[16] using home-collected and mailed specimens. With this approach, female adolescents are mailed a collection kit at home and asked to self-collect a vaginal specimen and then mail the test to a central laboratory. Their experience demonstrates that this approach can be highly successful in reaching a large number of untested individuals and identifying asymptomatically infected adolescent females.

Epidemiologic studies using NAATS tests have increasingly called attention to the high incidence of recurrent chlamydial urogenital infection, especially among adolescents with an initial chlamydial infection. In the Washington State chlamydia registry, for example, of 32,000 women treated for chlamydia from 1993 to 1998, 15% had one or more recurrences over 3 years.[17] Among those younger than 20 years, 17% recurred by 2 years. Importantly, only one third of these recurrences were actually seen at the same clinic, suggesting that studies of recurrence based at a single clinic may well underestimate considerably the true prevalence of recurrent infection. In a report describing the CDC multicenter cohort study to evaluate chlamydial recurrences in women, Whittington and colleagues[18] reported on a cohort of 1,194 young women treated for chlamydia and followed by LCR testing at 1 month and 4 months after initial therapy. Overall, 13% had recurrence at 4 months. They concluded that rescreening at 3 months posttreatment might be an excellent way to identify a high-risk group of adolescents infected with chlamydia. Burstein and colleagues[19] reported on both incident and prevalent chlamydial infections in an inner-city group of adolescent women studied at family planning, STD, and high school–based clinics utilizing urine LCR. Over 3,000 women were followed and the average follow-up was approximately 33 months. In their study, the time to an incident new chlamydial infection for those initially uninfected was about 7 months and the time to reinfection for those initially infected was approximately 6 months. They concluded that the very high chlamydia prevalence and incidence in these girls warranted more frequent screening, perhaps as frequent as every 6 months.

Until the NAATS era, virtually all recommendations for chlamydia screening were focused on women. Now, how-

ever, a rationale has emerged for chlamydia screening in young men.[20] First, there is a substantial prevalence of asymptomatic infection in adolescent men. Second, identification and treatment of such infections would constitute primary prevention for women. Third, identification and treatment of the asymptomatic male reservoir might help to prevent reinfection and recurrences in women. Finally, testing in men would also engage and better educate young men regarding STD prevention. One major obstacle that has prevented successful screening of young men in the past was insensitive laboratory tests that did not have the ability to detect the low inclusion count infections that frequently are present in asymptomatic men. In addition, tests utilizing urethral swabs have generally been unacceptable to asymptomatic males, many STD control programs could not afford to extend screening to males, and finally, gaining access to adolescent males who infrequently attend healthcare facilities essentially requires a test that can be used in an outreach program.

Although the need for increased chlamydia screening seems obvious to those in public health, there remain significant barriers to actually implementing such programs. First and foremost is cost. The best tests for screening, the NAATS tests, are unfortunately the most expensive tests. In Washington State, we have been monitoring the types of tests used for chlamydia screening throughout the state. In 1994, it was disappointing to learn that many laboratories testing for chlamydia were actually using the so-called rapid tests, which are the least sensitive tests available and are not intended for use in laboratories but rather in clinics and physician offices.[21] In a more recent 1999 survey, use of these tests has declined considerably among laboratories and NAATS testing is being implemented in more and more laboratories.[22] However, the use of some non-NAATS tests, in particular DNA probe tests and EIA tests, has actually increased and are now the most commonly used tests. Also somewhat disappointing is the realization that many physicians have not yet implemented routine chlamydia screening practices. In a study by Cook and colleagues[23] of a random sample of physicians in Pennsylvania accessed by mailed questionnaires, fewer than half of the physicians surveyed indicated that they would screen sexually active adolescent girls for chlamydia if given the opportunity. Depending upon their subspecialty, the proportion that said they would screen young women for chlamydia ranged from 28% among family practitioners to 49% among pediatricians. It is clear that important teaching messages are needed to facilitate active screening programs.

Additional information also is needed to strengthen the rationale for and demonstrate the beneficial effects of screening. At present, evidence suggests that screening young women for chlamydia will decrease their risk of PID.[24] In addition, introducing screening into a population of women will, over time, decrease the population prevalence of chlamydial infection and the occurrence of PID.[25] The effects of chlamydia screening on subsequent PID incidence were well demonstrated by our study, which was a randomized intervention trial of screening versus nonscreening among young women in a large health maintenance organization. The study demonstrated a substantial reduction in the subsequent incidence of PID among those screened. However, whether screening males results in any beneficial effects in either men or women is as yet virtually unstudied.

Chlamydial Persistence

Many people describe chlamydia as a persistent pathogen.[26] According to Casadevall,[27] persistence indicates a state of infection in which the host response does not eliminate the microbe, resulting in continuing damage over time. Persistence may evolve into overt disease, depending on the balance of the host-microbe interaction. Many observations suggest that chlamydia is indeed a persistent infection as Casadevall defined persistent. For example, in trachoma, both elementary bodies and chlamydia genes have been demonstrated in culture-negative individuals over long periods of time, even after patients have left endemic areas. Similarly, in Reiter's syndrome, chlamydial elementary bodies and genes have been demonstrated in affected joints, presumably carried there from the initial site of genitourinary infection via macrophages.[28] In addition, chlamydial genital infections can persist for months without treatment as has been demonstrated in a number of different circumstances, and culture-negative infections can be reactivated in some circumstances when patients receive immunosuppressive medication. Finally in cell culture systems, it is clear that interferon gamma, penicillin, or amino acid deprivation can induce culture-negative persistence of chlamydial infection characterized by overproduction of hsp-60 and downregulation of other chlamydia genes such as *omp1*.[29] Withdrawal of these factors results in viable chlamydia being seen once again.

An important issue in patients is whether the recurrences being seen, as alluded to previously, do in fact represent persistence, i.e., relapse of a prior inadequately treated chlamydial infection. Alternatively, the recurrences could be reinfections from a new or untreated sexual partner. Reinfection can be unambiguously distinguished from persistence or relapse only if the reinfection involves a new strain. Many reinfections, however, undoubtedly occur but are with the same strain as the original infection as assessed by molecular epidemiologic tools. An example of this dilemma is seen in the seven patients that we described at our center who were found to be infected on multiple occasions over a 3 to 5 year period with the very same serotype, and in fact the same genotype when assessed by DNA sequencing of the major outer membrane protein gene. At some

visits, these individuals were culture-negative but LCR positive and they then became culture-positive once again for the same strain.[30] Examples such as this would seem to indicate that long term persistence in a given individual does occur, but one cannot be certain that despite multiple rounds of antimicrobial treatment, a persistently infected partner did not serially reinfect such individuals.

It is possible that current treatment regimens are ineffective in some individuals and result in persistent long-term infection. In truth, there have not been many studies using long-term follow-up to assess individuals treated for urogenital chlamydial infection; almost all treatment studies have spanned only a 4-week follow-up period. It is possible that our treatment regimens only suppress chlamydial infection, allowing the infection to persist and subsequently relapse in some persons.

A second related question would be whether antimicrobial resistance in some chlamydial strains allows such persistence to occur. As noted above, few studies have followed treated individuals beyond 4 weeks after therapy to assess efficacy utilizing the most sensitive tools available. Workowski and colleagues,[31] however, did follow a cohort of women infected with chlamydia for 20 weeks after treatment with 7 days of doxycycline, seeing them at regular intervals and assessing test of cure by culture and PCR. Although a few individuals remained persistently positive by PCR at 2 weeks after completion of therapy, essentially none recurred with the originally infecting strain throughout the 20 weeks of follow-up. One individual was reinfected with a serologically different strain than the original infecting strain. It should be noted that these individuals were seen at a student health center and had relatively small numbers of sexual partners. The results of this study would suggest that doxycycline given for 7 days does not often result in microbiological or clinical failure. Also of interest would be similar studies using single dose azithromycin, which has become the standard of treatment and has not been evaluated in this manner.

Is it possible that we have failed to recognize antibiotic resistance among chlamydia urogenital tract isolates? Certainly not many patients are cultured, and even fewer undergo routine test of cure. There is essentially no ongoing surveillance for chlamydia resistance and only a relatively small number of strains associated with resistance phenotypes, i.e., isolates from individuals whose strains have been collected in association with clinical failure, have been studied. Finally, laboratory methodologies for evaluation of resistance are not standardized and methodological considerations could prevent recognition of resistance. Recently, clinical treatment failure associated with in vitro chlamydial resistance has been reported. Soso and colleagues[32] reported three patients with multiple positive chlamydial cultures despite receiving therapy. Two patients had clinical failures associated with apparent resistance to chlamydia as demonstrated in vitro. Further studies are needed to more broadly assess the relationship between resistance to antimicrobials as seen in vitro and clinical treatment failure or persistence.

In closing, let me reiterate my gratitude to the ASTDA for this recognition and for the many other opportunities it has provided me. Finally, my biggest thanks to my wife, Peggy, who has been a constant source of support, encouragement, and energy throughout my career.

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