Protective Immunity to *Chlamydia trachomatis* Genital Infection: Evidence from Human Studies

Byron E. Batteiger,1,2 Fujie Xu,3 Robert E. Johnson,3 and Michael L. Rekart4,5

1Department of Medicine, Division of Infectious Diseases, and 2Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis; 3Division of STD Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia; and 4British Columbia Centre for Disease Control and 5University of British Columbia School of Medicine, Vancouver, British Columbia, Canada

**Background.** Some screening and treatment programs implemented to control *Chlamydia trachomatis* genital infections and their complications have shown initial reductions in infection prevalence, followed by increases to preprogram levels or higher. One hypothesis is that treatment shortens duration of infection, attenuates development of protective immunity, and thereby, increases risk of reinfection.

**Methods.** A literature review was undertaken to assess evidence supporting the concept of protective immunity, its characteristics, and its laboratory correlates in human chlamydial infection. The discussion is organized around key questions formulated in preparation for the Chlamydia Immunology and Control Expert Advisory Meeting held by the Centers for Disease Control and Prevention in April 2008.

**Results.** Definitive human studies are not available, but cross-sectional studies show that chlamydia prevalence, organism load, and concordance rates in couples decrease with age, and organism load is lower in those with repeat infections, supporting the concept of protective immunity. The protection appears partial and can be overcome after reexposure, similar to what has been found in rodent models of genital infection. No data are available to define the duration of infection required to confer a degree of immunity or the time course of immunity after resolution of untreated infection. In longitudinal studies involving African sex workers, a group presumed to have frequent and ongoing exposure to chlamydial infection, interferon-γ production by peripheral blood mononuclear cells in response to chlamydial heat-shock protein 60 was associated with low risk of incident infection. In cross-sectional studies, relevant T helper 1-type responses were found in infected persons, paralleling the studies in animal models.

**Conclusions.** The data support the concept that some degree of protective immunity against reinfection develops after human genital infection, although it appears, at best, to be partial. It is likely that factors besides population levels of immunity contribute to trends in prevalence observed in screening and treatment programs. Future studies of protective immunity in humans will require longitudinal follow-up of individuals and populations, frequent biological and behavioral sampling, and special cohorts to help control for exposure.

Immunity capable of resolving a genital chlamydial infection and protecting against reacquisition of infection has been characterized largely in the mouse and guinea pig animal models, as reviewed in detail by Rank and Whittum-Hudson [1]. To date, it has not been considered to be safe and practical to experimentally infect humans with *Chlamydia trachomatis*, and thus, no human challenge studies are available. It is not ethically acceptable to withhold treatment for known infections. Therefore, we cannot define directly in humans the natural course of infection, the course of reinfection at various times after resolution of primary infection, or the effect of treatment on development of protective immunity. Because of these limitations, available evidence in humans of protective immunity is mostly indirect and inferred from epidemiologic studies.

Evidence that immunity is capable of resolving infection in humans has been found in limited studies...
of natural history, reviewed in detail by Geisler [2], which consistently showed that humans clear chlamydial genital infections in the absence of antibiotic treatment. Two studies involving women suggested that clearance after 1 year is 45%-55% [3, 4], reaching 94% by 4 years [4]. Another study involving pregnant women showed 44% spontaneous clearance within 2-3 months [5]. Two recent studies with much shorter follow-up periods, as well as a prior review, indicated that genital infections in male individuals also clear over time [6-8]. The data in aggregate strongly support the concept that immunity capable of resolving infection develops in humans. However, unlike in animal models, the time course to resolution in humans is measured in months to years rather than in weeks. Thus, these same studies clearly demonstrate that untreated infections may persist for many months and suggest that C. trachomatis is capable of evading host defenses and/or that an effective human response, in some cases, is slow to develop.

Our primary focus here is on human protective immunity, as measured by reduced detection of infection after reexposure after an initial infection or reduced organism burden or duration of shedding after reexposure. Defined in this way, protective immunity develops in response to prior infection and might be complete (no detectable infection after reexposure) or partial, characterized by shorter duration of organism shedding, lower organism burden, or both after reexposure. In animal models of genital infection, complete protection has been observed but very short-lived, whereas partial protection has been uniformly observed and longer lasting [1]. We were not able to find solid evidence for this short period of complete protection in human infection. Nevertheless, partial protection may be very important from a disease control standpoint, because shorter infection duration and quantitatively less organism shedding are probably associated with decreased risk of transmission. Epidemiologic studies that bear on the concept of protective immunity in the context of genital disease are the focus of our review. The role of immune responses in either promoting or protecting against development of upper tract disease in women is reviewed by Darville and Hiltke [9].

Although useful parallels are found between trachoma and genital infections, there are important differences in biology and epidemiology. Biologically, serovars A and C are strictly associated with trachoma (serovars B and Ba cause trachoma but also cause genital disease), are tropic for conjunctival epithelium, and differ from genital strains, because they lack an intact trpA gene and cannot use indole to synthesize tryptophan [10]. Other differences are found in genes encoding polymorphic membrane proteins [11], cytotoxin [12], and the translocated actin-recruiting phosphoprotein [13], but the relevance of these differences to pathogenesis is currently unclear.

Epidemiologically, trachoma transmission is driven by sharing of ocular secretions among young children in family and community groups, facilitated by poor facial hygiene and the ubiquitous presence of flies [14]. Exposure and reexposure in areas of hyperendemicity might be conceived of as more or less continuous. In contrast, exposure to sexually transmitted chlamydial infection is episodic and depends not only on behavior (coitus) but also on the probability that coitus occurs with an infected person. Despite these differences, recent studies related to immunity in trachoma are included in the review when they fill gaps or corroborate data from studies of genital infection. However, trachoma immunity is not the focus of the review, and trachoma vaccine studies are not reviewed.

Markers of essential mechanisms of immunity defined in animal models, such as chlamydia-specific CD4+ T lymphocytes, interferon (IFN)-γ, and immunoglobulin at mucosal sites, have been documented to exist in humans. These studies are of interest because they provide evidence of responses in humans that are analogous to effective responses defined in model systems, even if the responses have not been studied longitudinally to establish associations between them and protective immunity.

This review is organized around 4 key questions developed by the authors in preparation for the Chlamydia Immunology and Control Expert Advisory Meeting, held 23–25 April 2008. A literature review was undertaken regarding protective immunity in humans, specifically in the context of the contemporary epidemiology of chlamydial genital infections and the implementation and effectiveness of control programs.

**METHODS**

An Ovid Medline search was conducted by one of the authors (F.X.) in January 2008 for articles published since 1950 under the following major headings: immunology, immunobiology, or immune response; susceptibility, immunity, recurrent infections, repeat infections, subsequent infections, reinfections, or recurrence; and T lymphocytes or antibodies. The search was then narrowed to the terms “chlamydia” or “Chlamydia trachomatis” and “human” and to articles in English. After excluding articles dealing with infants and persons >65 years of age, the author reviewed a list of titles for 1287 articles for relevance, focusing on primary research articles rather than reviews. Among the articles evaluating laboratory parameters of immunity, the reviewer focused on those evaluating samples obtained from studies involving infected persons, with or without uninfected control subjects, and excluded articles focused primarily on in vitro studies using human cell lines.

Using the resulting list of 209 articles, 2 authors (F.X. and R.E.J.) conducted a second review for relevance and selected 108 articles for review in detail by all coauthors (R.E.J., M.L.R., F.X., B.E.B.). Summaries were prepared and assembled into tables of evidence relating to the key questions. The tables included study design and methods, outcome measures, find-
ings related to key questions, a judgment of strengths and weaknesses, and relevance to key questions. Additional articles found through searching reference lists of the identified articles and others found relevant by the panel of reviewers were included. After a summary of the tables was presented at the meeting, the most relevant studies were incorporated into this synopsis.

A limitation of this synopsis is that some informative articles may not have been reviewed. In addition, representative examples of articles presenting certain types of evidence are cited, rather than all such articles.

RESULTS

Evidence of Protective Immunity in Humans Infected with C. trachomatis

What evidence supports the concept that humans develop protective immunity after infection with C. trachomatis? If protective immunity develops, is it (1) partial or complete; (2) serovar, serogroup, or species specific; and (3) long-lasting or short-lived?

Association of age and chlamydial prevalence. One of the most robust epidemiologic characteristics of both genital and ocular chlamydial infection is higher prevalence among younger persons than among older persons. For example, the highest rates of genital infection are among women aged 14–19 years and men aged 20–29 years [15]. This inverse relationship between age and prevalence has been interpreted to suggest that protective immunity is acquired over time. However, in young women, the period of highest risk of infection also correlates with the period when cervical ectopy, a risk factor independently related to prevalence of chlamydial infection, is most frequent [16]. In addition, the 18–19- and 20–24-year age groups in both men and women are peak intervals for having ≥2 sex partners in the previous 12 months [17]. A strong inverse relationship of age and prevalence is also noted for gonorrhea [15], which may be behavior or development related, given that little protective immunity develops in response to gonorrhea because of extensive antigenic variation in gonococci. Therefore, it is difficult to distinguish the effects of development and behavior from those of acquired immunity in persons with chlamydial genital infection. A large (n = 14,605) retrospective cross-sectional culture-based study involving men and women at high risk of infection at a sexually transmitted disease (STD) clinic analyzed age in relation to sex, race, history of STD, physical findings, numbers of sexual partners per year, and frequency of sexual relations. In multivariate analysis, age was an independent inverse predictor of C. trachomatis culture positivity in both men and women [18]. Furthermore, when a subset of persons known to be exposed to C. trachomatis was analyzed in multivariate analysis, age persisted as an inverse independent predictor of chlamydial infection [18]. However, in this study, immune responses, as measured by microimmunofluorescence serologic examination and blastogenic responses of peripheral blood mononuclear cells (PBMCs) to chlamydial elementary bodies, were not correlated with age.

Similar age relationships have been identified in trachoma, but in prospective, longitudinal studies. Estimated prevalences and durations of infection and rates of incident infection were significantly higher among young children (age, 0–4 years) than among older (age, >15 years) children [19]. A more recent study not only confirmed the inverse age relationship of trachoma infection and disease but also suggests that higher levels of local IFN-γ messenger RNA transcripts are found in older persons [20]. Thus, in trachoma, the inverse association of age and C. trachomatis prevalence exists at a site anatomically and physiologically different from the genital tract. This suggests that age-dependent changes in genital tract physiology and sexual behaviors may not be the exclusive reasons for the observed association in epidemiologic studies of genital infection. The results [20] further associate relevant elements of the mucosal immune response, such as IFN-γ, with age in a manner consistent with acquired protective immunity.

Association of age and organism load. In addition to rates of chlamydial infection, organism load has been inversely related with age, suggesting that acquired immunity may restrict chlamydial replication in older persons. The largest study used quantitative cell culture at the endocervix in 1231 women at high risk of infection who attended an STD clinic [21]. In multivariate analysis, age was inversely correlated with higher inclusion counts. Although there is the potential for confounding with unmeasured developmental events, cervical ectopy was not associated with organism load in univariate analysis.

Association of STD history and chlamydia prevalence. Several studies have shown an association of higher C. trachomatis infection rates among persons who self-report no history of STD, suggesting that potentially naive persons are more susceptible because of lack of acquired immunity. A study involving 2546 men and 1998 women at high risk who attended an STD clinic showed a significantly higher culture isolation rate among those who self-reported no STD history [22]. A larger cohort (n = 14,605) derived from the same STD clinic also indicated that self-report of no STD history is associated with higher isolation rates [18]. In contrast, other studies have shown that documented history of chlamydial infection is associated with increased risk of current infection [23] and that self-report of past STD is a risk factor for incident infection [24].

Infection concordance in sexual partnerships. Study of chlamydial infection in sexual partnerships could provide evidence to support the concept of protective immunity, although many factors other than adaptive immunity may affect transmission. Low concordance of infection in sexual partnerships...
indicates the possibility of protective immunity preventing transmission. Quinn et al [25] used nucleic acid amplification tests (NAATs) to evaluate sexual partnerships in a cross-sectional study at STD clinics. Of 494 partnerships, NAAT results were negative for both partners in 79.6%. Of partnerships in which ≥1 partner was infected, only 1 partner was infected in 32% and both partners were infected (concordant infection) in 68%. Young age was a risk factor for concordant infection in both partners [25]. A smaller NAAT- and STD clinic–based study of 106 partnerships found 76% infection concordance [26]. A small contact-tracing study in Boston selected partnerships in which it was reasonable to infer the direction of transmission—in this case, from men to women. The concordance rate for NAAT-proven chlamydial infection was 65%. In the same study, the concordance rate for Neisseria gonorrhoeae infection, which confers little protective immunity because of extensive antigenic variation, was 73% [27]. Thus, in concordance studies in STD clinic populations, only modest support is found to suggest protective immunity. However, a community-based study in German gynecologic practices that comprised 1690 asymptomatic couples screened by NAAT [28] showed that, of 78 couples with ≥1 positive partner, only 27 (35%) were concordant. The participants tended to be older and in longer relationships than participants in the STD clinic–based studies. In addition, the rate of concordance decreased with age. Although statistical tests were not reported for this trend, the results are consistent with the possibility of protective immunity acquired with age.

**Studies of repeated infections.** Many studies, mostly involving women, have evaluated the timing and risk of repeated infections during a period of observation. These studies demonstrate another robust epidemiologic characteristic of chlamydial genital infections: repeated infections are common. The median time to reoccurrence is typically 5–7 months, and the risk of incident infection is higher among younger women than among older women [29, 30]. A study at school-based health centers reported a cumulative incidence of repeat infections of 26.3% in 1 year; another study at community-based health centers reported that 59.6% of women with initial infection had developed repeat infection at 18-month follow-up [31]. In a study of repeat chlamydial infection sought at scheduled follow-up visits ~1 and ~4 months after treatment of an index infection, the cumulative recurrence rate was 13.4% at 4.3 months. The factor associated most strongly with repeat infections was resumption of sexual activity [32]. Finally, a study involving sex workers indicated that the strongest epidemiologic predictor of incident infection is C. trachomatis infection at the baseline visit [33]. In aggregate, these observations do not exclude the concept of protective immunity, but they strongly suggest that it is partial at best.

**Serotyping or genotyping in repeat infections.** A few studies have evaluated repeat infections with use of serotyping or genotyping methods to help define the nature of repeat infection. As a biomarker, strain typing is most helpful when the infecting serovars or genotypes at the 2 episodes are different; this establishes that reinfection is likely to be responsible for the repeat episode. When the serovars or genotypes at each episode are the same, interpretation is less clear; such episodes could represent reinfection from an untreated partner or antibiotic treatment failure. Among 72 STD clinic patients, repeat infections occurred with the same serovar in 33.8% of cases when the expected rate of same-serovar recurrences based on the distribution of serovars was only 18.4% [34]. In a study of repeat infection in adolescent women, 48 had repeat infections with serovar determination at both episodes over a period of 21 months; 44.8% of the repeat infections were with the same serovar [35]. In both studies, it is probable that most of the repeat infections with the same serovar were reinfections, because the majority of patients had negative culture results between repeat infections. Brunham et al [36] found that, among sex workers, 13 (62%) of 21 repeat infections occurring 1–6 months after an index infection were the same genotype, whereas only 2 (11%) of 19 occurring >6 months later were the same genotype. The authors suggested that the low proportion of same-genotype infections at >6 months is consistent with serovar-specific immunity, although epidemiologic factors, such as partner change, were not examined. More recently, a larger study involving adolescent women comprising 183 infection pairs with complete genotyping at both episodes also found that early repeat infections were more likely to be caused by the same genotype than were late repeat infections; however, the late different-genotype infections were significantly associated with partner change [37]. On balance, over the short term, serovar-specific immunity appears not to have a clinically pronounced effect in genital chlamydial infection.

Data from a culture-based study involving STD clinic patients suggest that protective immunity may occur but is limited in duration [22]. In both men and women, a laboratory-documented chlamydial infection <6 months before an index visit was associated with a lower prevalence of infection than was a documented chlamydial infection >6 months earlier. Although suggestive of protective immunity that is limited in duration, it is possible that the more recent treatment in the <6-month groups may have reduced the number of prevalent infections detected at the index visit. Finally, a study using quantitative DNA amplification techniques in a small number of patients with repeat infections showed that organism load is lower in those with repeat infections, suggesting that prior exposure may restrict replication at the local site as a result of acquired but partial immunity [38].

**Studies involving sex workers.** Sex workers have frequent sexual interactions, and thus, the potential for repeated ex-
posure to \( C.\) \textit{trachomatis}–infected clients is substantial. Although studies involving sex workers are difficult to generalize to at-risk but non–sex worker populations, the data may allow detection of protective immunity in a setting where sexual activity is consistently high. A series of prospective studies involving sex workers in Nairobi has been undertaken to study a highly if not uniformly exposed population in which protective immunity might be detected and its correlates evaluated.

Brunham et al. [36] found in multivariate analyses that the probability of incident chlamydial infection was inversely related to duration of prostitution. Furthermore, increased risk of incident infection was associated with human immunodeficiency virus (HIV) status, independent of CD4+ T cell count. Both associations suggest that acquired immunity develops in this population and reduces acquisition of incident infection. The same cohort was used to study risk factors for chlamydial pelvic inflammatory disease (PID) [39]. In this analysis, the primary finding related to protective immunity is that, among HIV-infected women, those with an entry CD4+ T cell count <400 cells/mm³ were at highest risk of developing PID, suggesting that CD4+ lymphocytes are required to restrict chlamydial replication and prevent establishment of upper tract disease. This finding was followed up in a separate cohort enrolled on the basis of clinical diagnosis of PID; PBMCs from a subset of 95 women were evaluated for cytokine production in response to stimulation by whole elementary body antigens. IFN-γ production was lower in HIV-infected patients than in HIV-uninfected persons; furthermore, the responses were associated with lower CD4+ T lymphocyte counts [40]. These laboratory evaluations are consistent with the clinical correlations and suggest that T helper 1 (Th1) cell mechanisms are required to limit upper tract pathology.

Cohen et al. [33] reported incident chlamydial infections as a function of both epidemiologic and laboratory measures of immune responses in a cohort formed to study the immunoepidemiology of sexually transmitted infections in female sex workers. In this study, 299 women were enrolled and followed up prospectively for incident chlamydial infection. The major epidemiologic factors associated with risk of incident chlamydial infection included \( C.\) \textit{trachomatis} infection at enrollment, incident \( N.\) \textit{gonorrhoeae} infection, young age, and <2 years duration of sex work. In contrast to the earlier study [36], HIV-1 infection was not correlated with risk of incident chlamydial infection. The inverse association of young age and shorter duration of sex work and incident infection are consistent with the concept of protective immunity. However, baseline \( C.\) \textit{trachomatis} infection and incident gonococcal infection were strong risk factors for incident infection. These results suggest that these women were exposed to a subset of men with a higher prevalence of \( C.\) \textit{trachomatis} and that protective immunity is partial, although the prevalence of \( C.\) \textit{trachomatis} infection among the clients of the sex workers was not reported. The major laboratory correlate of decreased risk of incident infection was IFN-γ production by PBMCs stimulated by recombinant chlamydial heat-shock protein 60 (cHSP60) at baseline. Among 29 persons positive by this measure, no incident chlamydial infections were observed. The importance of this specific laboratory measure is supported by the results of another study in which PID and history of repeated chlamydial infection were associated with reduced IFN-γ production in PBMCs stimulated by cHSP60 [41].

**Summary interpretation, gaps, and research directions.**

Several lines of evidence indicate that some degree of protective immunity to reinfection develops in humans over time. Protective immunity is likely to be partial at best and can be overcome after reexposure, paralleling the experience in animal models. To the extent that data are available, there is little convincing clinical evidence that immunity is serovar or ompA genotype specific. The duration and strength of partial protective immunity in humans are uncertain. Prospective studies involving sex workers and trachoma suggest that Th1-type immune mechanisms, including CD4+ T lymphocytes and IFN-γ, are important components of acquired immunity in humans. Although definitive human studies are not available, the picture of human protective immunity that emerges from the literature correlates well with characteristics and mechanisms of partial protection defined in mouse and guinea pig models of genital infection; the short-term complete immunity observed early after resolution of infection in the rodent models has not been demonstrated in human studies.

The primary measures of partial immunity in animal models are diminished intensity of shedding (organism load) and decreased duration of infection. Human studies using chlamydia incidence as an end point are not fully capable of detecting and characterizing such partial immunity. To further characterize protective responses in humans, longitudinal studies are required that include baseline and subsequent measurement of identified candidate markers, such as IFN-γ production by cHSP60 stimulation of PBMCs, together with serial sampling for incident infection and determination of organism load. Scheduled periods of frequent noninvasive sampling performed prospectively may identify incident infection in real time [42, 43] and, perhaps, estimate its duration. Study of special cohorts (eg, those including sexual partnerships [dyads]) may provide an opportunity to assess candidate markers and their ability to predict incident infection given known exposure.

**Duration of Prior Infection and Susceptibility to Reinfection**

Does the duration of prior infection(s) determine susceptibility to reinfection? Conversely, does early abrogation of infection by treatment inhibit development of protective immunity?

Most of the aforementioned repeat infection studies share
the characteristic that index infections, of variable but mostly unknown duration, were treated, thus truncating the natural course of infection. This raises the question of whether treatment of first or repeat chlamydial genital infections hampers the development of protective immunity that might ordinarily develop if a long-lasting infection were to resolve spontaneously.

A study in the mouse model of genital infection with *Chlamydia muridarum* clearly showed that antibiotic treatment, given before immune responses are fully developed, attenuates the development of protective immunity [44]. Doxycycline, begun at 4 different intervals after inoculation for a primary infection (days 0, 3, 7, and 10), was given for 14 days. Treatment promptly terminated chlamydial shedding and prevented or reduced the frequency of hydrocele. The course of infection after rechallenge was longer, with higher organism shedding in all of the treatment groups, compared with animals allowed to clear the primary infection without treatment; the earlier that treatment was started, the more profound the effect. Measures of immune response (local immunoglobulin [Ig] A, serum IgG, and IFN-γ production by chlamydia-stimulated splenocytes) were correspondingly reduced.

It seems likely that a similar phenomenon would be observed if experiments of this kind were possible in humans. We know that chlamydia-specific responses of PBMCs [45], infection-associated endocervical T cell infiltrate [46], and presence of interleukin (IL) 12 [47] at the endocervix decrease or resolve within 3–4 weeks after antibiotic treatment of genital infection. The major unknown is how long humans must be infected before partial protection develops. In mice, it is a matter of just a few weeks. In humans, prevalent infections can have durations of a few weeks to several years, depending on when or if they are detected. The duration of incident infections depends on the frequency of screening and treatment. To our knowledge, there is no direct evidence defining the minimum duration of infection that confers a given degree of protection. As can be seen from the mouse experiments, longer duration of infection produces protective immunity but also allows the development of upper tract pathology in the majority of animals [1]. Although to a substantially lesser degree [3], the same is probably true in humans [6, 48].

Findings of 2 epidemiologic studies suggested that treatment for *C. trachomatis* infection is associated with increased rates of reinfection. In each study, the authors hypothesized that treatment hinders the development of protective immunity. Two additional studies showed that the prevalence of serum antibodies to *C. trachomatis* decreased during a period when case rates and seroincidence increased.

The first study evaluated repeat genital infections in the Vancouver metropolitan area [49]. This large study (33,917 infected individuals) was undertaken because reports of chlamydial infec-

A transmission model was presented, based on a set of assumptions regarding protective immunity and ranges of various parameters related to the duration of infection as a function of prior infection. A modeled control strategy that shortens duration of infection (eg, by prompt treatment) recapitulates the empirical observations. The study results are consistent with the hypothesis of a population phenomenon of reduced immunity, although alternative reasons for the observations have been suggested on the basis of limitations of population surveillance and screening data. These include increased screening coverage [50]; increased use of NAATs for diagnosis [51]; changes in proportions of screened individuals at high and low risk of infection; testing frequency; uncertainty in establishing reinfection rates [52]; and contemporaneous factors, such as behavior change related to the HIV epidemic [53].

The second study reported rates of reinfection after targeted azithromycin treatment in the context of trachoma [54]. In the analysis of reinfection, patients with NAAT-documented *C. trachomatis* infection at baseline were identified in Vietnamese communes where trachoma is endemic; the duration of infection before baseline testing was not reported. Two communes were given targeted treatment with azithromycin at baseline and 12 months. Treatment was not based on infection status, which was determined post hoc, but was given to those with clinically identified active trachoma and their household contacts. The targeted treatment strategy resulted in treatment of 11% of infected individuals in these communes, indicating that a substantial pool of infected persons remained. In the third commune, no systemic antibiotics were given. Reinfection rates were examined at 18, 24, and 36 months. At 36 months, significantly higher reinfection rates were observed among individuals in the communes where azithromycin was given, although the total number of individuals infected at baseline who actually received azithromycin appears to have been small. The authors attribute the higher rates of reinfection to impaired development of immunity.
A third study was based on the seroprevalence of IgG antibodies against *C. trachomatis* in Finland [55]. Serum samples were analyzed from 8000 women representing a subset of the Finnish Maternity Cohort serum bank, stratified by calendar year and by age of the women at the time of sampling. Sero-prevalence was measured in samples obtained during 1983–2003. Among women <23 years of age, seroprevalence decreased from 16.0% during 1990–1996 to 10.6% during 1997–2003. A similar decrease was observed among women 23–28 years of age during the same periods (from 19.1% to 12.5%). The authors note that the number of reports of chlamydial infections has increased in recent years; specific data available from the Finnish Statistical Database of the Infectious Diseases Register (http://www3.ktl.fi/) show an increase from 8031 cases in 1995 to 12,863 in 2003. An increase in case rates coupled with a decrease in seroprevalence is consistent with a population decrease in protective immunity, although many other factors could contribute to an increase in reported case rates, including increased coverage and frequency of screening.

Finally, a recent follow-up study using the Finnish Maternity Cohort showed that the rate of seroconversion was higher in the 3-year period during 2001–2003 than during the period 1983–1985 among women aged 23–28 years (odds ratio, 3.2, 95%; confidence interval, 1.1–8.7) [56]. However, considering all 3-year periods over the entire duration of the study, there was not a significant time trend in this age group (P = .10). There were no significant time trends in seroconversion among women <23 years of age. An increase in seroincidence coupled with a decrease in seroprevalence would be consistent with a population decrease in immunity; however, the reported seroincidence data are, at present, inconclusive.

**Summary interpretation, gaps, and research directions.**

No studies are available that establish the minimum duration of chlamydial infection in humans that is required to elicit protective immune responses. Likewise, no direct data are available to indicate the point during the course of human infection at which antibiotic therapy can hinder development of relevant protective responses. In the animal study, early treatment appears to be required (within 10 days), but because of the large difference between the durations of mouse and human genital infections, it is difficult to extrapolate the results in the model to human infection. Some measures of cell-mediated responses in humans diminish shortly after antibiotic treatment. Epidemiologic studies of reinfection rates, chlamydia case report trends, and seroprevalence and seroincidence trends suggest that intensified case finding and treatment in some locales are associated with an initial decrease followed by paradoxical increases in reported chlamydial cases and, perhaps, seroincidence. The decrease in seroprevalence rates in Finland provides evidence that conveniently measured immune responses at the population level are less prevalent during an interval with an increase in reported cases. A reasonable hypothesis is that prompt treatment blunts the protective response, which in earlier preprogram eras, developed after long periods of untreated infection. A study in mice establishes proof of principle in a model system. However, further study is needed to better understand the relative roles of other potential explanations in these prevalence and incidence trends and reinfection rates, with characterization of screening coverage, frequency of screening, and other relevant epidemiologic variables. In addition, longitudinal study of serological responses in selected cohorts may provide insight into the duration of serum antibody presence and their relationship to incident chlamydial infection.

**Immune Responses Elicited by *C. trachomatis* Infection**

What innate and adaptive immune responses are elicited by *C. trachomatis* infection? Of these, which are associated with protective immunity? Have markers defining a protective immunity phenotype among at-risk individuals been identified?

On the basis of data available from studies in animal models [1], the key elements of resolving and protective immunity include trafficking of chlamydia-specific CD4+ T lymphocytes to the genital site and production of Th1-related cytokines, including IFN-γ capable of restricting chlamydial growth, and the presence of neutralizing IgG antibody at the local site. From the animal models, it is clear that the number of CD4+ lymphocytes at the mucosal site decreases as infection is cleared and that this reduction is correlated with the return of partial susceptibility to reinfection. At the time of rechallenge, local antibiotics are likely to reduce the infectivity of any given inoculum by neutralizing some proportion of elementary bodies, restricting the early intensity of shedding. Rapid anamnestic recruitment of memory T cells to the mucosal site more rapidly clears those reinfections. Together, these mechanisms account for the observed partial immunity. Here, we review representative studies to establish that elements of these essential responses are observed in human infection. The studies reviewed in this section are mostly cross-sectional, and thus, in most instances, the measured responses cannot be directly linked to protective immunity, except as noted.

**Mucosal cellular and cytokine responses.** An early study demonstrated that (1) IFN-γ can be found in endocervical secretions, (2) endocervical levels were higher in *C. trachomatis*-infected than in uninfected women, and (3) plasma levels did not correlate with infection. The major significance of this work was to document an important Th1 cytokine at the site of infection in humans [57]. Findings of a more recent study suggested that women with recurrent infection had higher endocervical levels of IFN-γ than those with primary infection [58]. In a multivariate analysis of endocervical cytokines and clinical characteristics, levels of IFN-γ, IL-12, and IL-10 were...
significantly associated with endocervical infection with *C. trachomatis* (*n* = 17) [59]. In a study involving 396 female adolescents, endocervical secretions were assayed for IL-2 and IL-12; infected women had lower levels of IL-2 and higher levels of IL-12 than did uninfected women. In addition, these relationships were confirmed in 96 women who contributed paired samples, documenting the changes as women moved from the uninfected to the infected state or vice versa [47]. IL-12 is produced by dendritic cells and induces IFN-γ production by T cells, biasing toward a Th1 response.

A careful immunohistochemical evaluation of the human vagina and cervix established that T lymphocyte subsets, antigen-presenting cells, macrophages, and dendritic cells are most abundant in the endocervical transition zone [60], suggesting that the endocervix, the primary site for lower genital tract chlamydial infection, is also the major inductive or effector site for cell-mediated immunity in the lower genital tract. The data show that noninvasive sampling via cytobrush or other cell collection techniques is useful in evaluating local responses to chlamydial infection. In a flow cytometry study of endocervical cells, CD4⁺, CD8⁺, and CD83⁺ (dendritic cell) phenotypes were more frequently present in infected women than in uninfected women, but CD19⁺ (B cell) phenotypes were not different. There also was no difference in PBMC phenotypes between infected and uninfected women [61].

In a recent, carefully conducted study, 20 infected women had samples obtained by cytobrush at the endocervix, both at the time of infection and 1 month after antibiotic therapy [46]. At the time of infection, accumulation of neutrophils and of CD4⁺ and CD8⁺ T lymphocytes was observed. Both during infection and after treatment, the predominant endocervical cell infiltrate was CD45RO-expressing effector memory T cells. Endocervical T cells also expressed CD103, consistent with mucosal homing. HLA-DR expression by T cells was significantly increased during infection, indicating activation. After treatment and documented clearance of chlamydiae, CD3⁺ cells were markedly reduced. There were no differences in PBMC phenotypes between infected and uninfected women, with the exception of a higher proportion of chemokine (C-C motif) receptor 5–expressing T lymphocytes after treatment.

In summary, these studies indicate that local Th1 cytokines, mainly IFN-γ, are associated with *C. trachomatis* infection and that an infiltrate of T lymphocytes predominates at the local site. The T lymphocyte infiltration is largely cleared within 1 month after treatment [46]. These findings are consistent with the changes associated with infection and clearance in both the mouse and guinea pig models.

**Mucosal and systemic antibody responses.** Local antibodies derived from persons with trachoma are capable of neutralizing chlamydial infectivity in animal models [62]. In addition, presence of antibody in local secretions, particularly IgA, was found to correlate inversely with quantitative culture of samples from the endocervix [63]. Cross-sectional studies have noted high rates of serum IgG antibodies to whole elementary bodies among STD clinic attendees, but in a large study, serological responses were not associated with age or reduced isolation rates of *C. trachomatis* [18].

Although IFN-γ production by PBMCs in response to chSP60 was significantly associated with protection against incident chlamydial infection in sex workers, endocervical IgG and IgA to whole elementary bodies or chSP60 were not [33]. In addition, serum IgG antibodies to whole elementary bodies and chSP60 were not associated with a lower risk of incident chlamydial infections.

Many contemporary studies of antibody bear on the risk of sequelae of chlamydial infections, such as PID [39], PID recurrence, and lower pregnancy rates [64]. Relationships between chlamydial serology and upper tract sequelae are reviewed elsewhere in this supplement by Haggerty et al [65] and Darville and Hilts [9]. Serological responses have been documented to other chlamydial proteins, as measured by immunoblotting [66], and to specific proteins, such as plasmid protein pgp3 [67] and chlamydial proteasome or protease-like activity factor [68]. However, these responses have not been examined in longitudinal studies in relation to incident chlamydial infection.

In summary, antibody responses, including those measured in endocervical secretions, have not been found to correlate with protective immunity but appear to be markers of prior infection. Certain antibodies to chSP60 or high titers of antibodies to whole elementary bodies appear to correlate with sequelae. Major unanswered questions include how long serum IgG antibodies persist after an episode of infection, whether disappearance of such antibodies is a marker of increased susceptibility to infection, and whether early treatment of infection prevents development of a serological response or shortens the period during which antibodies are detectable.

**Systemic cellular responses.** Although mucosal cellular responses are most relevant because of the epithelial location of infection [69], careful studies of systemic cellular responses have been made and are reviewed briefly. A notable series of articles by investigators at the DeMars laboratory have defined HLA class I and II–presented T cell epitopes in the major outer membrane protein (MOMP) of *C. trachomatis* in STD clinic attendees with genital chlamydial infection. MOMP epitopes that activate HLA class II–restricted T cells from humans with genital infections have been defined [70]. Human genital tract infections were also found to induce HLA class I–restricted CD8⁺ cytotoxic T lymphocytes specific for MOMP [71]. Most of the epitopes were found in constant sequence regions of the MOMP and were species specific. Subsequently, 5 MOMP peptides represented in variable sequence regions (where serovar-
specific B cell epitopes reside) were found to contain HLA class II–presented T cell epitopes that are serovar specific [72]. Finally, these authors used HLA-A2 tetramers to characterize MOMP-specific cytotoxic T lymphocyte responses and found MOMP-specific T cells in peripheral blood of infected but not uninfected persons [73]. These findings were then applied to a clinical study of trachoma, in which the frequency of MOMP-specific CD8+ T lymphocytes in peripheral blood was sought. The frequency of these cells correlated with active ocular infection, but sample sizes were too small to relate to protection or disease outcome [74].

Markers defining a protective immunity phenotype. At present, the only documented marker of protective immunity was that established by Cohen et al [33]. Specifically, IFN-γ production by PBMCs stimulated by chSP60 was found in a subset of 29 women in a longitudinal study of chlamydial incidence among Nairobi sex workers. None of these women acquired incident chlamydial infection during a study period of 24 months, a significantly lower rate than seen among women without such responses. Although a larger group of women had production of IFN-γ by PBMCs stimulated by whole elementary bodies, the rates of incident chlamydial infection were similar to those among women without such responses. It is possible that the chSP60 responses in a subset of these women were masked by responses to nonprotective T cell antigens represented on whole elementary bodies.

Nevertheless, the finding that PBMC production of IFN-γ in response to chSP60 stimulation is associated with decreased risk of incident infection is also consistent with findings in sex workers with PID. HIV-infected sex workers—known to have an increased risk of chlamydial PID, compared with women who are HIV uninfected—have a lower frequency of IFN-γ production in response to stimulation with C. trachomatis antigens [40]. In the latter study, PBMCs were stimulated with either whole elementary bodies or MOMP isolated by detergent extraction from elementary bodies. Future longitudinal studies in humans should clearly include similar assays using sets of these antigens (whole elementary bodies, MOMP, and chSP60) to confirm the associations found in populations of African sex workers.

Host or Organism Factors that May Affect Susceptibility to Reinfection

Host factors. Several studies that examined HLA alleles in the context of PID associated with chlamydial infection [39, 75, 76] are reviewed by Darville and Hiltke [9] and are not additionally considered here. Two articles summarize selected host factors in adolescents enrolled in the Reaching for Excellence in Adolescent Care and Health study, a longitudinal study with periodic biological sampling [77]. Similar to findings of other studies, young age, multiple partners, and prior chlamydial infection predicted incident C. trachomatis infection at follow-up. In addition, HLA class II allele DQB1*06 and HLA class I haplotype B*44-Cw*04 were associated with incident chlamydial infection [78]. The second article focuses on recurrent infections in a subset of 90 adolescents with repeat documented chlamydial infections separated by a negative assay result [79]. In multivariate analyses, HLA class II variants DRB1*03-DQB1*04 and DQB1*06 were associated with recurrence, whereas IL-10 promoter variants were underrepresented in those with recurrent infections.

Other than genetic factors, associations have been reported between chlamydial infection, quantitative chlamydial shedding at the endocervix, and concomitant gonococcal infection [21, 34, 36]. The basis for this association is not clear, although gonococcal IgA1 protease has been suggested as a biological factor [36]. In a cross-sectional study, bacterial vaginosis has been identified as a risk factor for acquisition of chlamydial and gonococcal infections in women recently exposed to a partner with urethritis [80]. Women harboring hydrogen peroxide–producing lactobacilli were less likely to be infected by either organism. Because the altered microbiota of bacterial vaginosis frequently include indole-producing anaerobes, some have hypothesized that the increased risk of chlamydial infection in bacterial vaginosis is related to the ability of genital strains to synthesize tryptophan from indole, thereby in part evading the activity of IFN-γ at the infected site [81].

Organism factors. The primary organism factor that has been evaluated in the context of human infection is serovar determination, either by immunoassay in older studies or, more recently, by direct sequencing of the ompA gene from clinical samples. Serotyping or genotyping studies performed in the context of repeated human infections are reviewed above [34–36]. Other serotyping or genotyping studies have been focused largely on establishing strain distributions in various populations and locales and associating clinical phenotypes with ompA polymorphisms. Studies involving sex workers indicate considerable strain variability found in variable sequence regions [36, 82]. Overall, serovars E, D, F, and Ia are seen most frequently, with some regional variations in the United States [83–85]; similar distributions are also noted in Europe, Asia, and Australia [86–89]. The classic disease-causing groups of C. trachomatis (trachoma, genital, lymphogranuloma venereum strains) and the associated tissue tropisms do not correlate with serovar orompA genotype [83, 85]; however, sequence variations in some polymorphic membrane protein genes do [11]. Newer multilocus genotyping methods have been described, but clinical correlations are limited to date [90–93].

SUMMARY

There are many obstacles in human immunology research. Unlike in animal models, human populations are genetically di-
verse, and even a carefully selected cohort will probably include a heterogeneous collection of those who have little or no protective immunity, various degrees of partial immunity, and various levels of exposure to infected partners. However, our synopsis supports the view that a degree of partial protective immunity develops as a result of genital chlamydial infection. In addition, the data suggest that IFN-γ production by PBMCs in response to chHSP60 represents a phenotypic marker of protective immunity for future studies. Our review also confirms the view that longitudinal studies of persons at risk that use serial collection of behavioral and biological data are necessary to further define elements of protective immunity and the responsible mechanisms. In the era of programs for screening and treatment of at-risk young persons, there is concern that prompt diagnosis and treatment increase risk of subsequent reinfection. It is not clear whether the observed increases in reinfections are related to changes in levels of immunity in the populations studied or related to epidemiologic and ascertainment factors related to screening and treatment. Additional research on the role of protective immunity in explaining these findings could help to optimize current strategies until effective vaccines for primary prevention of chlamydial infections are available.

Acknowledgments

We thank Sami Gottlieb and Stanley Spinola for helpful discussion and critical review of the manuscript.

References


52. Miller WC. Epidemiology of chlamydial infection: are we losing ground? Sex Transm Infect 2008; 84:82–86.


70. Ortiz L, Demick K, Petersen JW, et al. Chlamydia trachomatis major outer membrane protein (MOMP) epitopes that activate HLA class II-