SUPPLEMENT ARTICLE

Summary: The Natural History and Immunobiology of *Chlamydia trachomatis* Genital Infection and Implications for Chlamydia Control

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In 2008, the US Centers for Disease Control and Prevention held the Chlamydia Immunology and Control Expert Advisory Meeting to foster a dialogue among basic scientists, clinical researchers, and epidemiologists studying genital *Chlamydia trachomatis* infection. The objectives of the meeting were to determine key questions related to *C. trachomatis* natural history and immunobiology, with implications for control programs; to review existing data on these key questions; and to delineate research needs to address remaining gaps in knowledge. The 9 articles in this supplement to *The Journal of Infectious Diseases* describe salient findings presented at the 2008 meeting, and this commentary summarizes and synthesizes these articles and discusses implications for chlamydia control efforts and future research priorities.

Genital *Chlamydia trachomatis* infection is an important public health concern. The most common bacterial sexually transmitted infection in the United States and worldwide [1–3], chlamydia can lead to serious reproductive tract sequelae, including pelvic inflammatory disease (PID), tubal factor infertility, and ectopic pregnancy [4]. Because of this, many countries have initiated chlamydia control programs [5, 6]. However, as described in the introduction to this supplement of *The Journal of Infectious Diseases*, substantial, continuing decreases in rates of *C. trachomatis* infection have not been observed after implementation of chlamydia control efforts [7]. This somewhat unexpected scenario has sparked interest in reassessing what is known about the natural history and immunobiology of *C. trachomatis* infection and the implications for current chlamydia control strategies.

Because most genital *C. trachomatis* infections are asymptomatic, chlamydia control programs are based primarily on screening for asymptomatic, prevalent infection in young sexually active women, with varying emphasis on efforts to treat male sex partners and to screen women for reinfection. The assumptions underlying these programs are that they will reduce the number of adverse outcomes of chlamydial infection by (1) identifying infected women and treating them before the infection progresses to clinically relevant tubal inflammation or damage (secondary prevention) and/or (2) reducing transmission of *C. trachomatis* in the population and, thereby, reducing the number of new incident chlamydial infections and their associated sequelae (primary prevention). However, the potential for current control efforts to reduce adverse reproductive outcomes through either primary or secondary prevention is heavily dependent on the natural history of genital *C. trachomatis* infection.

On an individual level, the effectiveness of chlamydia screening depends, in part, on the risk and timing of tubal inflammation and damage relative to acquisition of infection and the mean duration of infection at the time of screening. In addition, the benefits of averting

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subsequent tubal damage from detected infections must be weighed against susceptibility to new repeat infections and the risk of sequelae from those repeat infections. The impact of efforts to treat sex partners and to screen for reinfection depends, in part, on the degree to which protective immunity develops after an initial infection and whether tissue-damaging immune responses are accelerated with repeat infection. On a population level, generalized screening can shorten the mean duration of infection and has the potential not only to reduce the number of complications in infected women but also to reduce transmission and, thus, the number of new infections. However, these benefits must be weighed against a potential increase in the number of repeat infections and their attendant sequelae, especially if little attention is paid to treating male sex partners or screening for reinfection. Such a calculation depends, in part, on the relative harm of persistent infection, compared with new repeat infection, and the potential for protective immunity to develop and to be abrogated by treatment.

Thus, a better understanding of the natural history and immunobiology of genital *C. trachomatis* infection could dictate the optimal structure of a chlamydia control program. Such information could guide, for example, how resources should be allocated for screening asymptomatic women, compared with treating sex partners of those infected; the optimal frequency of screening; or the optimal intensity of efforts to rescreen previously infected women. In 2008, the US Centers for Disease Control and Prevention (CDC) held the Chlamydia Immunology and Control Expert Advisory Meeting to (1) determine the key questions related to *C. trachomatis* natural history and immunobiology, with implications for control programs; (2) review how existing data shed light on these key questions; and (3) delineate research needs to address remaining gaps in knowledge. Answers to these questions may help guide future control efforts to prevent the adverse health effects of genital *C. trachomatis* infection. The articles in this supplement to *The Journal of Infectious Diseases* describe in detail the salient findings presented at the CDC meeting [8–16]. This commentary summarizes and synthesizes these articles and discusses implications for chlamydia control efforts.

PATHOGENESIS

Because the ultimate goal of chlamydia control programs is to prevent reproductive tract complications, the first step in approaching the intersection between *C. trachomatis* immunobiology and control is to understand how chlamydial infection leads to sequelae. In this issue, Darville and Hiltke [10] describe 2 hypotheses for chlamydial pathogenesis—the cellular paradigm and the immunological paradigm—and the lines of evidence for each. Aspects of both of these processes may play roles in pathogenesis, and a better understanding of the relative importance of each in leading to adverse outcomes could help shape control programs. Under the cellular paradigm, pathogenesis is driven primarily by inflammatory responses initiated and propagated by host epithelial cells, the primary targets of chlamydial infection [17]. The infected epithelial cells secrete chemokines, which recruit inflammatory leukocytes to the site, and cytokines, which induce and augment the cellular inflammatory response [17, 18]. Inflammatory mediators from both infected host cells and infiltrating immune cells induce direct damage to the tissues. Ongoing release of inflammatory mediators during chronic persistent infection or repeated responses with repeated infections could lead to cellular proliferation, tissue remodeling, and scarring. According to the immunological paradigm, pathogenesis is primarily the result of adaptive cellular immune responses directed at specific chlamydial antigens during repeat or persistent infection [19]. The chlamydial-specific adaptive T cell responses that develop over time to help clear infection are thought to induce collateral tissue damage or, if they fail to clear infection, to orchestrate inflammatory pathology during ongoing chronic infection.

Darville and Hiltke [10] maintain that pathogenesis is dependent on ascension of chlamydiae from the cervix to the fallopian tubes, and they emphasize the importance of gaining a better understanding of what facilitates and predicts such ascension and how to prevent it. If the innate responses of infected epithelial cells are sufficient to drive pathology, then tissue-damaging responses could begin to occur as soon as the fallopian tube is infected and would continue throughout the course of active infection. Thus, control programs should focus on preventing new infections and detecting existing infections as soon as possible after acquisition. If adaptive chlamydial-specific cellular responses mainly cause disease, tissue damage would mainly occur later in the course of an initial infection, after meaningful cell-mediated immune responses have developed. With repeat infection, this process could be substantially accelerated and augmented, and even small amounts of chlamydiae in the fallopian tubes might provoke an enhanced T cell response. Thus, efforts could focus on screening women with longstanding infection, but particular attention would need to be paid to preventing repeat infection.

How do we determine the relative importance of one pathogenesis paradigm over the other? Much of the evidence related to the innate response to *C. trachomatis* infection and the cellular paradigm has come from abundant in vitro and mouse model data. Darville and Hiltke [10] outline the usefulness of these models in determining potential mechanisms for development of genital tract tissue damage independent of T cell responses (eg, the critical role for chlamydial activation of the innate immune receptor, Toll-like receptor 2, subsequent inflammatory cell influx, and release of tissue-damaging proteinases from activated neutrophils) [20, 21]. A wealth of data has also shown that interferon (IFN)–γ-producing $CD4^+$ T helper

type 1 (Th1) responses develop over time during primary infection and play a central role in clearance of chlamydial infection [14, 22, 23]. Data on the immunological pathogenesis paradigm come mainly from guinea pig and, especially, nonhuman primate models, in which limited tubal damage is noted during primary infection, but adaptive cell-mediated immune responses are enhanced during repeat chlamydial infection and are associated with enhanced tubal damage [10, 24, 25].

A number of limitations exist in extrapolating data from various animal models to humans. A fundamental difference in the natural history of chlamydial infections between humans and, especially, rodent models is the duration of primary infection, which Miyairi et al [14] describe in detail in this supplement. Inoculation of mice with *Chlamydia muridarum* or guinea pigs with *Chlamydia caviae* generally leads to a self-limited genital infection with a rapid peak, plateau, and then rapid elimination; detection of viable chlamydiae from the lower genital tract is limited to 3–4 weeks [14]. In nonhuman primates, such as the pigtailed macaque, infections are more chronic and indolent in nature. Peak infection may not occur for months, and intermittent shedding of *C. trachomatis* can occur for up to 15 weeks [14, 26]. In another article in this supplement, Geisler [11] describes the limited human studies on the duration of untreated genital *C. trachomatis* infection. These studies demonstrate that the probability of infection resolution increases over time, with about half of chlamydial infections spontaneously resolving ~1 year after initial testing and, conversely, half of infections persisting [11, 27]. Human natural history studies to date have major limitations, including the absence of precise data on the timing of infection acquisition, limited use of *C. trachomatis* strain typing methodologies to confirm persistent as opposed to new infection, and the limited generalizability of findings from various study populations. Ethical considerations make these types of studies difficult to conduct. Nonetheless, available studies show that the duration of chlamydial infection in humans is on the order of months to years rather than weeks.

The differences in the duration of untreated infection between humans and animals may be related to differences between humans and animals in the innate and adaptive immune responses to chlamydial infection. On the basis of animal models, the key elements of the immune response effecting resolution of infection include trafficking of chlamydia-specific $CD4^+$ T cells to the genital site; production of Th1-type cytokines including IFN-γ, which inhibits intracellular chlamydial replication; and presence of IgG antibody at the genital site, which can inactivate extracellular chlamydial elementary bodies (EBs) [14, 15]. In women, $CD4^+$ T cells are indeed recruited to the cervix during active infection; however, $CD8^+$ and dendritic cells are also recruited, and the relative proportions of these cells may be situational [28, 29]. Several studies involving women have documented local Th1 cytokines, mainly IFN-γ, during active chlamydial infection, although these studies have been unable to determine which specific responses lead to infection resolution versus persistence [11, 30–32]. In addition, IFN-γ–mediated effector mechanisms may differ between animals and humans. IFN-γ–inducible effectors in mice include p47GTPases, and a primary effector in humans appears to be indoleamine dioxygenase, which limits the ability of *C. trachomatis* to synthesize needed tryptophan from indole [33]. Thus, the polymicrobial environment of the female genital tract, including the indole-producing anaerobes associated with bacterial vaginosis, might allow evasion of IFN-γ activity in some women [34]. Serum and genital mucosal IgA and IgG antibodies to specific *C. trachomatis* proteins and to chlamydial EBs are usually detected during active infection in women [11, 35–37], but their precise role in resolution of infection remains unclear. To gain a better understanding of which immune responses predict persistent infection versus clearance of infection in humans, both Geisler and Miyairi et al [11, 14] call for more translational research to apply what has been learned thus far from animal models to human studies. Use of "humanized" animal models or nonmurine models in which prolonged infection can be established may also be useful [14].

Because of the ability of chlamydiae to cause chronic persistent infection in humans, a key question is whether and how *C. trachomatis* might evade the immune system over long periods but continue to induce immunopathogenesis. One factor that may be at play is raised by Wyrick in this supplement [16]. Wyrick describes the normal developmental cycle of chlamydiae, in which infectious but metabolically inactive extracellular EBs cycle to noninfectious but metabolically active intracellular reticulate bodies (RBs) and then cycle back to EBs with release back into the extracellular milieu. In vitro, an aberrant RB phenotype occurs in response to various inducers, where the developmental cycle is stalled in a state characterized by "viable but noncultivable chlamydiae involving morphologically enlarged, aberrant, and nondividing RBs" [16]. The condition is reversible to yield infectious EBs on removal of the inducers. Inducers of the aberrant RB phenotype include IFN-γ, penicillin, iron deprivation, nutrient starvation, maturation of host cells into physiologically differentiated states, and concomitant herpes simplex virus infection [38, 39]. Although all persistence-inducing conditions can exist in vivo, it is still unknown whether aberrant chlamydial RBs occur in vivo and, if so, whether they contribute to chronic inflammation, fibrosis, and scarring. To address this issue, Wyrick [16] urges that more translational research be done, using such techniques as electron microscopy, confocal microscopy, and molecular studies of inclusions in biopsy specimens from women selected from clinically well-defined cohorts.

Clearly, the balance between inflammation leading to infec-

tion clearance and inflammation leading to pathology is an important consideration in understanding the natural history of *C. trachomatis* infection. In general, $CD4^+$ T cells modulate the in vivo immune response to an infection by differentiating into 2 distinct subsets. Th1 cells primarily enhance the cell-mediated immune response to intracellular pathogens through synthesis of proinflammatory Th1 cytokines, especially IFN-γ and interleukin (IL)–12. Th2 cells primarily enhance the humoral immune response to extracellular pathogens and regulate the Th1 response through synthesis of antiinflammatory Th2 cytokines, especially IL-4 and IL-10 [40]. Human studies of the cellular immune response to *C. trachomatis* infection are limited but mainly show that human mucosal lymphocytes and peripheral blood mononuclear cells (PBMCs) skewed toward Th1 rather than Th2 predominance (ie, producing high levels of IFN-γ and low levels of IL-10 after stimulation with chlamydial antigens) are protective against sequelae [41–43]. However, nonhuman primate studies in particular have provided some evidence for the potential double-edged nature of the T cell response, linking Th1-type responses with pathology [25]. The specific immune responses and cytokine levels that lead to resolution of infection rather than promotion of tissue damage remain undefined, and pathogenic responses may be more complicated than simple Th1 versus Th2 T cell polarization. A more recently defined CD4 T cell lineage, Th17, which has been implicated in several other immunopathological disorders [44], might explain how ongoing inflammation could cause pathogenesis without adequate clearance of *C. trachomatis*. The Th17 cytokine profile is proinflammatory and recruits activated neutrophils to the site of infection but does not include IFN-γ. Thus, such lineages could cause pathological inflammation without mediating clearance of intracellular chlamydiae [45]. Further study of the role of Th17 cells in *C. trachomatis* pathogenesis and a better understanding of the immune response parameters most predictive of disease in humans remain important research priorities. Although logistically very difficult, more prospective studies involving women with *C. trachomatis* infection, including assessment of cellular and cytokine profiles and delineation of immune response dynamics, are essential.

Another critical question related to chlamydial pathogenesis is what proportion of women infected with *C. trachomatis* develop pathological complications. In a guinea pig model, tubal infection occurred in ~80% of guinea pigs after vaginal inoculation; however, after 1 infection, less than half had any kind of tubal damage, and only 12% developed chronic hydrosalpinx [46]. Analogous data on the proportion of women with *C. trachomatis* infection who develop tubal infection do not exist, but in an article in this supplement, Haggerty et al [13] attempted to assess the risk of sequelae after untreated chlamydial infection in women through a review of epidemiologic studies. Although numerous case-control studies have demonstrated the association between evidence of past chlamydial infection and either infertility [47–49] or ectopic pregnancy [50, 51], the authors found no prospective data directly assessing rates of long-term reproductive complications after untreated *C. trachomatis* infection. Some data were available, however, on the risk of symptomatic PID associated with untreated *C. trachomatis* infection and on the risk of long-term outcomes after PID.

In their review of symptomatic PID after untreated infection, Haggerty et al [13] describe 3 studies in high-risk settings, such as sexually transmitted diseases clinics and emergency departments, in which 2%–4.5% of women with untreated *C. trachomatis* infection developed PID within the ~2-week interval between testing and returning for treatment [52–54]. However, in 2 studies involving women at lower risk who had untreated infection and were followed up prospectively over longer periods, investigators did not observe proportionally higher percentages of PID diagnoses [55, 56]. In a small study involving 30 women with untreated chlamydial infection, no women developed PID over 1 year [55]. In another, 4 (3.7%) of 109 asymptomatic adolescent girls with untreated infection reported a hospitalization for PID or an emergency department visit for lower abdominal pain and vaginal discharge during 3 months of follow-up [56]. All of the reviewed studies were relatively small and had major limitations that could affect the accuracy of risk estimates. Natural history studies are inherently difficult, because it is unclear how long a woman has already had infection at the time it is detected through testing, and the standard of care is treatment of chlamydial infection after it is diagnosed. Another fundamental problem relates to outcome measurement. Clinical diagnosis of PID is notoriously insensitive and nonspecific [13] and may be dependent on clinician practices in a given setting. For example, clinicians may have a lower threshold for PID diagnosis in high-risk settings or if they know a patient has untreated infection. Synthesis of data across studies is also limited by the populations studied and tests used. For example, women in populations at high risk are more likely to have concurrent coinfections or a history of chlamydial infection or PID. Use of highly sensitive nucleic acid amplification tests in some studies may detect *C. trachomatis* infection with a lower organism burden and perhaps a lower probability of progression. In addition, women may seek care in high-risk settings, such as sexually transmitted diseases clinics, earlier in the course of a new chlamydial infection because of recent high-risk behavior. Thus, higher rates of symptomatic PID in these settings may be attributable to higher rates of symptomatic PID early in the course of chlamydial infection.

After symptomatic PID of any cause has occurred, ~1 in 6 women will develop infertility [57, 58]. In a landmark study conducted from 1960 through 1984, 1844 women with clinically suspected PID underwent laparoscopic examination and were

followed up for several years for adverse outcomes [58]. A key finding was that severity of salpingitis as determined by laparoscopic examination was linked to subsequent infertility risk in a dose-response fashion, suggesting that the intensity of inflammation during acute infection predicts long-term fibrosis and scarring, even with treatment. Of all women with clinically suspected PID, 26% had no laparoscopic evidence of salpingitis; only 3% of these women developed infertility, and none had confirmed tubal factor infertility. In contrast, 16% of women with laparoscopically confirmed salpingitis subsequently developed infertility, including 11.1% with confirmed tubal factor infertility [58]. A more recent longitudinal study involving US women with clinically suspected PID found that 18% developed infertility over the subsequent 3 years [57]. Although PID, regardless of etiology, is linked to adverse outcomes, data from the largest studies suggest that *C. trachomatis*–associated symptomatic PID is no more or less likely to lead to sequelae than other causes of PID [59, 60].

Several lines of evidence also suggest that *C. trachomatis* infection can lead to long-term reproductive complications, such as infertility, without symptomatic PID as an intermediary event. First, asymptomatic upper tract chlamydial infections have been documented [61]. Second, most women with tubal infertility do not have a history of symptomatic PID, even in studies showing strong associations between infertility and serologic evidence of past chlamydial infection [47, 62]. Finally, pathological damage in tubal biopsy specimens from women with tubal factor infertility is similar with or without a history of diagnosed PID [63]. Thus, subclinical tubal infection with *C. trachomatis* and consequent inflammation may lead to infertility and other complications in a significant number of women; however, no published studies have directly evaluated this in a prospective fashion, and the full extent to which this occurs remains unclear.

To gain a better understanding of the risks of sequelae after untreated *C. trachomatis* infection, Haggerty et al [13] emphasize the importance of developing innovative, standardized methods to more accurately measure acute PID and subclinical tubal involvement associated with chlamydial infection. Newer, noninvasive measures of tubal inflammation and damage should be explored as advancements are made in laboratory methods and radiological techniques (eg, magnetic resonance imaging or power Doppler ultrasound) [64, 65]. Ultimately, additional prospective studies are needed on the risk of clinically suspected PID, subclinical tubal inflammation, and long-term tubal damage resulting from untreated *C. trachomatis* infection in diverse populations, including women in the general population currently targeted by control programs. Because of the aforementioned limitations, such studies will be challenging, and creative new approaches will be needed. Haggerty et al [13] suggest that genital specimens from prospective studies of other infections (eg, human immunodeficiency virus prevention trials and human papillomavirus vaccine trials) might provide opportunities for evaluating the natural history of chlamydia. Studies of *C. trachomatis* natural history must be carefully designed to ensure adherence to ethical standards.

Although risk estimates are not precise and many gaps in knowledge remain, it is nonetheless clear that most women with *C. trachomatis* infection do not develop PID, and most women with PID do not develop infertility or other long-term complications. Thus, there must be additional microbiological and/or host factors that contribute to pathogenesis. In this supplement, Byrne [9] discusses potential virulence properties of *C. trachomatis* and how they may relate to pathogenesis. Traditionally, strain distinctions have been made primarily on the basis of variations in the chlamydial major outer membrane protein gene (*ompA*) [66, 67]. However, differences in genital *C. trachomatis* strains, as defined by *ompA* variation, have not been linked consistently with differences in disease severity or clinical presentations [9]. Byrne [9] describes a number of other candidate factors that might more accurately distinguish chlamydial strains with respect to pathogenic potential on the basis of their likely functional characteristics. These include the polymorphic outer membrane autotransporter family of proteins (Pmps) [68], type III secretion system effectors [69], and the putative large cytotoxin [70]. Ultimately, Byrne [9] emphasizes the critical importance of expanding the definitions of chlamydial strains beyond the major outer membrane protein paradigm to better understand virulence properties and how these properties might reflect disease severity. Continued work on development of a chlamydial gene transfer system and the application of genomic approaches to large collections of well-characterized clinical isolates may aid in identifying important virulence factors. The association between specific chlamydial gene products and disease outcomes cannot be interpreted without considering many factors, including host genetics, history of infection, and the hormonal and polymicrobial milieu at the time of infection [71, 72]. Thus, complex data sets, including both host and pathogen factors, will likely be needed, as will innovative new biostatistical analytic approaches [9].

PATHOGENESIS BIOMARKERS

Because of the likely role of both innate and adaptive immune responses in pathogenesis, it is not surprising that genetic variation in host responses may play a role in determining why some women develop pathology and others do not. Various genetic determinants, such as HLA class I and II variants and functional polymorphisms in cytokine and cellular receptor genes, have been assessed in relation to chlamydia-related outcomes in disparate populations [10, 73–75]. However, it has been difficult to clearly define specific alleles or polymorphisms that reliably predict pathology because of the complex nature

of the immune response, the likelihood of finding associations by chance when evaluating a large number of potential determinants, potential linkage disequilibrium with closely related determinants, and the generalizability of findings given the populations evaluated [74]. Unbiased genome-wide delineation of important human genetic determinants of sequelae would enable a better understanding of chlamydial pathogenesis and could also lead to development of useful biomarkers. Noninvasive markers that could reliably predict increased risk of complications would be extremely valuable, not only for the optimization of natural history studies, but also for targeted public health strategies that, for example, identify women who need more frequent screening or more intensive follow-up of sex partners. Biomarkers that could reliably predict susceptibility to incident and recurrent infection would have similar public health implications [11, 76, 77]. More detailed evaluations of host immune responses against a wider range of chlamydial antigens and use of newer high throughput DNA sequencing technologies to screen a larger number of genetic determinants may add insight [11, 78]. Such approaches will rely on rapidly evolving advancements in genomics, transcriptomics, proteomics, and bioinformatics [78, 79].

Researchers have attempted to identify clinically useful biomarkers by using currently available technologies. For example, vaginal neutrophil defensin levels, a measure of neutrophil activation, have been associated with the presence of endometritis in a cross-sectional study [80]. However, the precise role of defensins in the innate immune response to *C. trachomatis* ascension and in predicting tubal pathology has not been determined. Serological markers have also been assessed. Studies have consistently shown that women with adverse reproductive outcomes, such as infertility or ectopic pregnancy, are more likely to have chlamydia-associated antibodies or higher titers of these antibodies than are women without these outcomes [47, 48, 50, 51]. Some but not all studies show that serum antibodies predictive of sequelae frequently recognize *Chlamydia* heat shock protein 60 (cHSP60) [75, 81], an antigen known to be up-regulated during in vitro chlamydial persistence [16]. Among women with a history of *C. trachomatis* infection, the proportion with anti-cHSP60 antibodies increases in parallel with increasing severity of clinical disease manifestations [19]. However, these antibodies may simply be markers of greater exposure to chlamydiae (through either persistent or repeated infection), which is in turn associated with pathogenesis, rather than implicating these antibodies in pathogenesis of disease [10]. Chlamydial antibodies may also be markers of a Th2-weighted cellular response in certain women who, thus, are less likely to have a protective Th1 response. To gain a better understanding of the usefulness of serologic tests as markers of cumulative exposure to chlamydiae and predictors of disease, prospective studies should assess the proportion of *C. trachomatis* infections resulting in seroconversion, the time course of seroconversion, the duration of seroreactivity, changes in antibody titers with initial and repeat infection, and associated clinical outcomes.

REPEAT INFECTION

Another important factor that may determine why some women develop sequelae and others do not is the number of *C. trachomatis* infections that they acquire. Guinea pig and nonhuman primate models show that T cells infiltrate infected tissue more rapidly and in larger numbers and are associated with greater tissue destruction and fibrosis during repeat chlamydial infections, compared with initial infection [25, 82, 83]. Data from the macaque salpingeal pocket model, in which fallopian tube tissue was grafted to the abdominal skin in subcutaneous pockets, have suggested that the enhanced inflammatory response during repeat infection may be mediated by cytotoxic $CD8^+$ T cells primed against cHSP60 [84, 85]. These animal model data have been widely interpreted as meaning that repeat infections are inherently more dangerous than initial infections (ie, that risk of tubal damage per infection is not constant but rather increases with each additional infection). However, the animal studies have a number of limitations. For one, studies on repeat infections in macaques have mostly used direct inoculation of fallopian tubes [82] or salpingeal pockets [85], which does not necessarily mimic natural sexually acquired ascending infection. With direct inoculation, potentially damaging memory T cells home directly to the fallopian tubes, whereas in sexually acquired infection, there is time for these cells to home to infection at the level of the cervix and, thus, potentially resolve infection before it ascends. In addition, in nonhuman primate studies, repeated exposures were often given every 2–4 weeks, without treatment between exposures, even though natural infection in these primates may last several months [25, 82]. This model does not resemble the human situation in which a woman with a detected and treated infection or with a naturally resolving infection is reinfected later, often after many months or even years. However, this animal model may parallel the situation in which a woman with *C. trachomatis* infection is repeatedly inoculated by an infected partner over the course of one sexual relationship. If repeated inoculation is more often associated with ascension of the organism to the upper tract and pathology in humans, this could have important prevention implications. For example, condom use might provide additional benefit beyond primary prevention of sexually transmitted infections if it reduces risk of PID caused by repeated exposures among women already infected with *C. trachomatis* [86].

Available human epidemiologic studies have shown that the cumulative risk of PID [75,87] and long-term reproductive consequences [87, 88] increases in parallel with the number of

repeated *C. trachomatis* infections. Repeated *C. trachomatis* infections may also explain findings from 2 large prospective studies showing that women with at least 1 detected and treated *C. trachomatis* infection have higher rates of PID from any cause in the ensuing years than do women without a detected infection [89, 90]. However, it remains unclear from the available studies whether the risk of sequelae per infection increases with each additional repeat infection [75]. Thus, although a woman with 2 infections likely has a greater risk of sequelae than does a woman with 1 infection, it is unknown whether the cumulative risk is simply additive (the same risk with each infection) or more than additive (greater risk of sequelae during each subsequent infection). In some studies of repeat infections, clinicians' knowledge about prior positive *C. trachomatis* test results may influence subsequent diagnosis of lower abdominal pain as PID. It is also difficult to determine whether a first diagnosed infection is truly primary, the number and timing of past infections when there is evidence of past infection, and whether women with no detected infections have truly never had chlamydial infection. In all of the published studies, past infections were treated. It is possible and, perhaps, even likely that pathologic immune responses may differ after infections that resolve on their own, compared with those that are iatrogenically terminated. Additional studies assessing repeated *C. trachomatis* infections are needed to better characterize the nature of the cellular and humoral immune response during reinfection and the natural history of these infections, particularly the risk of adverse reproductive consequences in women. In addition, the high rates of PID from any cause during the years after a detected chlamydial infection indicate a need for studies of prevention strategies focused on women who have already received a diagnosis of at least 1 infection.

Numerous studies have shown that repeat chlamydial infections are common. In a systematic review of repeat *C. trachomatis* infection based on data from the most rigorous prospective studies, the peak reinfection rate was estimated to be ~20% at 1 year among women [91]. Surveillance data from British Columbia show that the number of repeat infections has been increasing over time, which likely contributes to observed increases in reported cases of *C. trachomatis* infection in that Canadian province [92]. It would be expected that, as more previously tested women are retested in a control program, the number of repeat infections will increase as a proportion of all detected infections. However, Brunham et al [92, 93] proposed an "arrested immunity" hypothesis to explain increasing numbers of reported chlamydia cases in several regions during an era of expanding chlamydia control efforts [1]. They postulated that shortening the mean duration of *C. trachomatis* infection through early detection and treatment by control programs has led to population-wide reductions in protective immunity and, thus, a marked increase in the number of repeat infections [92, 93]. Clearly, explanations other than arrested immunity could explain the observed epidemiologic trends. For example, increased screening coverage and frequency and increased use of more-sensitive diagnostic tests can lead to an increase in the number of reported chlamydia cases, even when there has been no true increase in the burden of genital *C. trachomatis* infection [94, 95]. In the United States, *C. trachomatis* infection burden, as demonstrated by national prevalence estimates, has not been increasing despite a steady increase in the number of chlamydia case reports [1, 96, 97]. Nonetheless, the arrested immunity hypothesis raises fundamental questions about whether and to what extent women develop protective immunity against reinfection with *C. trachomatis* and whether it can be abrogated by treatment.

PROTECTIVE IMMUNITY

In this supplement, Rank and Whittum-Hudson [15] review the evidence for development of protective immunity in animal models. Protective immunity to reinfection can be complete (ie, no organisms can be detected at the site of inoculation after reexposure) or partial (ie, organisms can be detected at the site of reinoculation, but there is a shorter duration of organism shedding and/or a lower organism burden after reexposure than during initial infection). In animal models, evidence strongly supports development of protective immunity; however, immunity against reinfection is complete only in the short term [15]. For example, guinea pigs are completely immune to reinfection 1–2 weeks after resolution of primary infection, but all animals become infected when challenged ~6 weeks later [98]. This short-term complete immunity is likely related to presence of antigen-specific T cells, which begin to decrease rapidly as soon as chlamydial antigen is no longer present [99]. Partial protective immunity, on the other hand, exists for a much greater duration (eg, >2 years in guinea pigs) [100]. IgG antibody, which unlike T cells, persists in the genital tract through constant transudation from serum, likely reduces the peak level of a reinfection through neutralization of EBs, and a rapid anamnestic T cell response then abbreviates the duration of the reinfection [15]. With regard to the effect of treatment on protective immunity, a published study of a mouse model clearly showed that antibiotics given at varying times up to 10 days after primary infection can attenuate development of protective immunity [101]. However, it is difficult to extrapolate these results to humans because of the marked differences in the durations of natural infection between mice and humans. Rank and Whittum-Hudson [15] also describe other reasons why animal models used to date might not parallel human infections and how to design better studies. For example, rodents have typically been inoculated at 2 discrete times (once for primary infection and once for repeat infec-

tion), whereas in humans, sexual activity with an infected partner may occur multiple times in a given time frame.

In addition, in this supplement, Batteiger et al [8] review the evidence for the development of protective immunity in humans. Several cross-sectional studies have demonstrated that younger age is associated with higher prevalence of chlamydia, higher organism load, and a higher degree of concordant infection status between sex partners [102–104]. These studies attempted to control for sexual behavior, cervical ectopy, and other potential confounding factors; thus, age associations were more likely to reflect immunity acquired over time. However, it was not possible to completely eliminate all confounding. A prospective study involving a cohort of Kenyan sex workers confirmed the inverse association of incidence of chlamydia with age and with duration of sex work; however, baseline *C. trachomatis* infection was nonetheless a strong predictor of subsequent chlamydial infection [105]. Another study assessing a small number of individuals with repeat infection found that organism load was lower during repeat infections than during initial infections in the same patients [106]. Several studies have provided evidence of human immune responses that are analogous to those resulting in partial immunity as defined in animal models, including chlamydia-specific $CD4^+$ T cells, Th1-type cytokines (mainly IFN-γ), and immunoglobulin at mucosal sites [8]. However, it is important to note that longitudinal studies of these responses in humans are sparse; thus, their association with protective immunity to genital tract chlamydial infection remains undefined. Small studies involving women that have evaluated IFN-γ production by PBMCs stimulated with cHSP60 have suggested that this cytokine may be important in protection against incident or repeat chlamydial infection [43, 105]. Limited data also show that treatment of human *C. trachomatis* infection rapidly diminishes the magnitude of the cellular immune response [8, 32, 107].

Batteiger et al [8] also summarized recent epidemiologic assessments that might provide clues about whether arrested immunity has a tangible impact on reinfection rates. For example, investigators in Finland found that seroprevalence of IgG antibodies against *C. trachomatis* decreased significantly among women between 1990–1996 and 1997–2003, although the number of reports of chlamydial infections increased during the same period [108]. A true increase in case rates coupled with a decrease in seroprevalence is consistent with a population decrease in protective immunity [92]. However, it is critical to recognize that an increased number of case reports does not necessarily reflect a true increase in incidence of *C. trachomatis* infection. In addition, in this study, women with repeated infection could contribute to case rates more than once but to seroprevalence only once. In a follow-up study involving the same Finnish population, seroconversion rates among paired serum samples (and, thus, seroincidence) were assessed [109]. No significant trends over time were observed from 1983 through 2003, although the authors found that seroincidence was higher during 2001–2003 than during 1983–1985 among women 23–28 years of age (but not younger women) [109]. Thus, it remains unclear whether there is truly an inverse association between population-based measures of immune responses and rates of new infection.

Taken together, the available data support the idea that some degree of protective immunity develops in humans; however, protection appears partial at best and can be overcome upon reexposure. Nevertheless, even partial immunity could affect transmission dynamics on a population level. Batteiger et al [8] emphasize the need for future prospective studies to better characterize protective immune responses in humans and the effect of antimicrobial treatment on altering these responses. Such studies will likely require measurement of identified candidate markers, such as IFN-γ production by cHSP60 stimulation of PBMCs, and serial sampling to detect incident infection and determine organism load. Frequent, prospective noninvasive sampling could identify incident infection in real time and allow better assessment of duration of infection [110]. Couples studies, which prospectively evaluate sexual partnerships (dyads), may provide a unique opportunity to assess factors predicting incident infection in the context of reasonably well-defined sexual exposure histories.

The reviews by Rank and Whittum-Hudson [15] and by Batteiger et al [8] assessed protective immunity with respect to presence of the organism, organism load, and duration of infection during reinfection, but not with respect to development of pathology. This is important because partial immunity to *C. trachomatis* may exist in humans; however, reinfection, even of relatively short duration, might elicit an even stronger pathologic response. For example, in guinea pig models, repeat infections were markedly shorter and had reduced bacterial burdens, compared with primary infection; however, more animals with repeat infection developed tubal dilatation [24]. Trachoma studies have shown that a protective immune response reduces the ability to isolate chlamydiae in the context of an ongoing pathologic immune response elicited by a small amount of antigen [111]. During genital tract infection in women, 2 fundamental components are necessary for pathology to develop: ascension of infection to the fallopian tubes and an immunopathologic response to infection (whether innate and/or adaptive) in the tubes. A more vigorous partial protective immune response could, on the one hand, reduce infectious burden and more quickly resolve infection at the level of the cervix and, thus, reduce likelihood of ascension to the upper tract. On the other hand, a more vigorous response could increase immune-mediated pathogenesis if and when infection has reached the upper tract.

BENEFITS OF SCREENING

Even if detection and treatment of a prevalent infection does interfere with development of protective immunity, the risk of tubal damage from potential repeat infection needs to be balanced against the benefit of eliminating ongoing prevalent infection. Epidemiologic data strongly suggest that a woman with 2 detected *C. trachomatis* infections has a greater risk for sequelae than does a woman with 1 such infection. However, in available studies, the duration of these infections is unknown. The absolute number of infections may simply be a reflection of a longer cumulative duration of infection. The key question is whether, for example, a woman with 2 infections of 6 months duration each has a greater risk for sequelae than a woman with 1 infection of 12 months duration. This depends not only on the nature of the immune response to tubal infection in initial versus repeat infections, but also on the risk of ascension of infection to the upper genital tract per unit time and the risk of tubal damage in the upper tract per unit time. If risk of ascension to the upper tract is constant over time, the difference in complication risk between 1 long infection and 2 short infections of equivalent cumulative duration depends primarily on the nature of the pathologic immune response during initial versus repeat infection. If there is a higher probability of ascension earlier in the course of infection (eg, before adaptive immune responses have limited infection to the cervix), repeat infections might be more harmful, even if the cumulative duration of infection is shortened.

The benefit of a chlamydia control program for an individual woman also depends on when pathogenic events occur relative to the timing of screening and how well treatment given at different times during the course of infection prevents adverse outcomes. In this supplement, Gottlieb et al [12] review studies on the benefits of screening to prevent sequelae among infected women. The authors identified only a few controlled trials directly evaluating the benefits of screening in prevention of PID and none directly evaluating the effect of chlamydia screening on long-term reproductive outcomes, such as ectopic pregnancy or tubal factor infertility. In a study designed as a randomized controlled trial involving 2607 young, high-risk women in a Seattle-area health maintenance organization, women receiving a 1-time invitation for chlamydia screening had an ~50% reduction in PID over the subsequent year, compared with a control group not invited for testing [112]. A cluster randomized trial of 1-time chlamydia screening in 17 Danish high schools also demonstrated a halving of PID occurrence over 1 year that was associated with screening [113]. However, both of these trials had methodological issues that may have affected the magnitude of observed screening benefits and might limit the generalizability of these findings to real-world settings [12]. For example, in the Seattle study, only 7% of the 36,547 initially randomized women were ultimately enrolled, and more aggressive efforts to contact women from the intervention group who did not respond to the initial eligibility survey may have introduced a selection bias and compromised randomization [12, 112]. In the Danish study, outcome assessment was unblinded, and almost 50% of participants were lost to follow-up [113]. A large, nonrandomized cohort study of *C. trachomatis* screening among >28,000 female US Army recruits did not find a substantial reduction in hospitalizations for PID among women who were screened, compared with those who were not [114]. Historical cohort and ecological studies have often been cited as evidence of the effectiveness of screening, but methodological limitations restrict valid conclusions.

Additional studies of the individual benefits of chlamydia screening would be valuable; however, study design is complicated by the degree to which screening programs are already established in a given area (ie, standard of care issues) [12]. New studies of screening strategies could provide an opportunity to incorporate much needed assessments of the natural history and immunobiology of *C. trachomatis* infection. For example, data from a randomized trial of chlamydia screening in the United Kingdom revealed that 9.5% of 74 asymptomatic, college-aged women with untreated infection developed PID in 1 year [115]. This is likely to be one of our best overall estimates of PID risk after chlamydial infection in a general population. However, final data from this trial were published too late for inclusion of a full critical review in this supplement.

Several population-level epidemiologic assessments have attempted to provide insight into the benefits of screening programs and whether repeat infections are more important in causing pathology than ongoing persistent infections. Although chlamydia control efforts have not been followed by substantial, continuing decreases in the burden of *C. trachomatis* infection as expected [7], published ecological data suggest that rates of PID and, perhaps, longer-term reproductive outcomes have decreased in the era of chlamydia control [45, 116–118]. If these ecological data represent a true cause and effect association, shortening the cumulative duration of infection may be more important in reducing chlamydia-associated complications than the potential risk of increasing the absolute number of infections (ie, persistent infection might play more of a role in chlamydial pathogenesis than repeat infection). However, ecological data need to be interpreted with caution for several reasons. Outcomes such as PID are difficult to measure accurately and are multifactorial. *C. trachomatis* infection may cause less than one-third of cases [119]; thus, changes in PID outcomes may be related to other factors, such as decreases in rates of *Neisseria gonorrhoeae* infection. Several ecological assessments have compared chlamydia surveillance reports with hospital discharge outcome data [45, 118]. Decreases in hospitalization rates for PID and ectopic pregnancy may simply

reflect a shift in care to the outpatient setting during the same period [116, 120]. In addition, there may be a delay of several years in observing increases in long-term outcomes, such as infertility and ectopic pregnancy, because even when tubal damage has occurred, it may not become apparent until an affected woman attempts to become pregnant. Furthermore, interpretation of surveillance data based on reported *C. trachomatis* infections is inherently problematic, especially in settings where screening coverage is low. In the United States, only ~40% of eligible sexually active women enrolled in health plans were screened for chlamydia in 2007, and this likely represents an overestimate of the national picture, because all of these women had health care visits [121]. We need better epidemiologic data to assess the benefits of chlamydia control programs, including assessment of the prevalence and incidence of chlamydia. In addition, there is a need for better measures of screening coverage and repeat infections, perhaps incorporating serologic testing to assess the latter. Validated systems that can more accurately capture and measure chlamydia-associated outcomes, including tubal factor infertility, on a large scale—not just limited to passively collected discharge or diagnosis codes—would also be valuable.

PROGRAMMATIC IMPLICATIONS

It has become clear that gaining a better understanding of the interplay between *C. trachomatis* immunobiology and chlamydia control strategies is essential. Although we currently have no evidence that control programs are not achieving their goals of reducing chlamydia-associated reproductive sequelae, many questions remain about the extent of that benefit and how chlamydia control programs should ideally be structured to maximize it. The articles in this supplement highlight several key questions related to *C. trachomatis* natural history, pathogenesis, and immunobiology that have implications for chlamydia control programs [8–16]. The natural history of *C. trachomatis* infection clearly involves a complex interplay between the organism and its host, characterized by the potential for both chronic, persistent infection and frequent reinfection. However, the precise mechanisms and degree to which human genital *C. trachomatis* infections resolve or persist, cause pathology, and stimulate immunity against reinfection have not been determined. A better understanding of these aspects of *C. trachomatis* immunobiology would have implications not only for the effectiveness, cost-effectiveness, and optimal structure of chlamydia control programs, but also for development of an effective chlamydia vaccine.

Insight into whether innate or adaptive immune responses are more important in chlamydial pathogenesis and the risk and timing of tubal inflammation and damage after acquisition of infection would have several programmatic implications. For example, this knowledge could help determine the optimal frequency of screening and rescreening and whether a program should focus primarily on detecting and treating long-standing prevalent infection or on reducing incidence of new infection in the population. A program focused primarily on reducing the incidence of new infections through interruption of *C. trachomatis* transmission might put greater emphasis, for example, on treatment of sex partners. Determination of specific cytokine and cellular responses that predict sequelae could eventually allow control efforts to be intensified for women at particularly high risk. Noninvasive markers of tubal inflammation and damage would also enable clinical trials to more accurately assess the benefits of control efforts. The long delay in observing important chlamydia-associated reproductive outcomes, such as infertility, has long hampered efforts to find the most effective control strategies. Greater understanding of the duration of infection in humans and the factors that predict infection resolution versus persistence is also critical. This would allow modeling the mean duration of infection at the time of screening and the number of self-limited infections that may be missed during a given screening interval. Evidence for a role of the aberrant RB phenotype in vivo could have implications not only for the effectiveness of current treatment strategies, but also for our ability to detect potentially important chronic infections through screening. Finally, a better understanding of the degree to which protective immunity develops in humans and whether control programs have any tangible effect on immunity at a population level is important, not because programs would withhold treatment of infection in an effort to enhance immunity, but to optimally model transmission dynamics to identify the best control strategies. This may affect the relative emphasis of a program on preventing incident and repeat infections through partner treatment and rescreening efforts, as opposed to relying solely on identification of women with long-standing infection. Such work would also have important implications for vaccine development.

RESEARCH NEEDS

To address remaining gaps in knowledge related to chlamydia immunobiology with implications for chlamydia control, several research needs have become apparent, as detailed in the individual articles in this supplement [8–16]. An overriding theme in all of these articles is the urgent need for more translational work, carefully planned prospective studies to better elucidate the natural history of *C. trachomatis* infection in humans, and development and validation of diagnostic tools and biomarkers to perform these types of studies. Examples of high-priority research needs are outlined in Table 1. Innovative translational studies are needed to determine how mechanisms of clearance, pathogenesis, and immunity found in animal and in vitro studies play a role in humans. Likewise, animal models should be refined to more closely parallel human exposure and

Table 1. Research Needs Related to *Chlamydia trachomatis* Immunobiology with Implications for Chlamydia Control

Research area	Translational research[a]	Prospective studies[b]	Diagnostic tools and biomarkers[c]
Infection clearance and persistence	• Determine role of chlamydia-specific $CD4^+$ T cells, IFN-γ–producing Th1 responses, and immunoglobulin at mucosal sites in resolving infection in humans • Investigate evidence for the aberrant RB phenotype in human biopsy specimens • Refine animal models that establish prolonged chlamydial infection	• Assess the duration of natural infection and rate of resolution of infection: include (1) collection of better information on timing of infection acquisition and (2) strain typing methodologies to distinguish persistent from new infection • Evaluate immune responses and host factors predictive of infection resolution vs persistence	• Define cellular and humoral markers associated with duration of infection • Develop strain typing methodologies that better distinguish persistent vs new chlamydial infection • Refine genomic, transcriptomic, proteomic, and bioinformatic approaches to identify a larger number of possible determinants of infection clearance
Pathogenesis	• Characterize inflammatory immune responses in humans and delineate relative contribution of innate and adaptive responses in pathogenesis: include correlation of cellular and cytokine profiles with pathology • Correlate host genetics, infection history, and hormonal and polymicrobial milieu during infection with disease outcomes • Evaluate association of candidate chlamydial virulence factors with disease severity, moving beyond the MOMP paradigm of strain distinction	• Evaluate the risk and timing of chlamydial ascension to the upper genital tract and immune responses predictive of ascension • Determine risk and timing of development of clinically important tubal inflammation and damage from untreated chlamydial infection • Assess association of repeated infections with sequelae in women • Conduct comparative trials of screening strategies to reduce sequelae • Evaluate strategies to prevent sequelae in women after at least 1 diagnosed chlamydial infection (eg, counseling, rescreening) • Model relative importance of persistent infection vs repeated infections in causing sequelae in populations with varied risks of repeat infection	• Develop and validate new accurate, noninvasive measures of clinical and subclinical tubal infection, inflammation, and damage: include newer radiologic techniques (eg, MRI, power Doppler ultrasound) • Develop standardized, validated algorithms for measuring PID, ectopic pregnancy, and tubal factor infertility on a population level • Define cellular and humoral markers predictive of ascension to upper genital tract, pathogenesis, and sequelae • Use new genotyping methodologies to define chlamydial virulence characteristics that may predict disease severity
Protective immunity against reinfection	• Determine role of chlamydia-specific $CD4^+$ T cells, IFN-γ–producing Th1 responses, and immunoglobulin at mucosal sites in complete or partial protective immunity in humans: include assessment of (1) immune responses associated with concordance of chlamydial infection between sex partners and (2) other factors altering protective immune responses (eg, infection duration, coinfections, host factors, antibiotics) • Develop animal models of protective immunity that more closely parallel human exposures • Identify chlamydial antigens selectively inducing protective immunity for vaccine development	• Evaluate specific immune responses in humans to prevent or attenuate reinfection (ie, reduce duration or organism load): include (1) assessment of IFN-γ production by cHSP60 stimulation of PBMCs and (2) frequent sampling to assess incidence, duration of reinfection, and organism load • Assess dynamics of cellular and humoral immune responses over time, with and without repeat infection	• Define cellular and humoral markers of protective immunity (complete or partial): would allow (1) measurement immunity in populations to model its role in determining burden of infection and (2) use in candidate vaccine trials • Refine genomic, transcriptomic, proteomic, and bioinformatic approaches to identify a larger number of possible determinants of protective immunity

NOTE. This table does not include a comprehensive list of all research areas that may advance understanding of *C. trachomatis* immunobiology and control, but rather outlines selected examples of research needs highlighted and described in more detail in the text. There may be substantial overlap and interdependence among research topics across rows and columns in the table; studies could be designed to incorporate components from several categories. Finally, feasibility and logistical constraints may vary across research needs and over time (eg, development of valid noninvasive biomarkers of duration of infection and of tubal damage would make natural history studies feasible across a wider range of study designs). All studies of *C. trachomatis* immunobiology and natural history must be carefully designed to ensure adherence to ethical standards. cHSP60, *Chlamydia* heat shock protein 60; IFN, interferon; MOMP, major outer membrane protein; MRI, magnetic resonance imaging; PBMCs, peripheral blood mononuclear cells; PID, pelvic inflammatory disease; RB, reticulate body; Th1, T helper type 1.

[a] Translation of findings from animal and in vitro studies to better characterize *C. trachomatis* infection in humans.

[b] Longitudinal epidemiologic evaluations to better elucidate the natural history of *C. trachomatis* infection in humans.

[c] Development and validation of accurate measures to assess and predict outcomes of *C. trachomatis* infection.

infection. We also need better prospective human data to provide insight into the duration of natural infection, the risk and timing of chlamydial ascension to the upper genital tract and of clinically important tubal damage, and development of protective immunity after initial and repeated *C. trachomatis* infections. Such studies should incorporate correlative assessments to determine immune response parameters, as defined in translational research, that are most predictive of these outcomes. Additional studies of the effectiveness of various screening strategies would be valuable; these studies could also help to better elucidate the natural history of *C. trachomatis* infection. Conducting research on the immunobiology of *C. trachomatis* infection in humans will be very challenging, and rigorous attention to ethical standards must be maintained. However, if carefully planned and executed with appropriate oversight by human research committees, such research is possible and should be a priority. Finally, to perform new translational research and prospective studies to inform chlamydia control programs, development of more accurate, noninvasive measures of tubal inflammation and damage to assess the out-

comes of *C. trachomatis* infection are crucial. Identification of immunologic biomarkers and other predictors of persistence, pathogenesis, and protective immunity would be important not only for research and vaccine development, but also as clinical tools to enable targeted control efforts. New studies of *C. trachomatis* immunobiology and control will be most fruitful if investigators across disciplines proactively explore opportunities to collaborate in addressing critical gaps in knowledge.

CONCLUSIONS

With many chlamydia control programs at a crossroads, research on the natural history and immunobiology of *C. trachomatis* infection is both an urgent mandate and also an exciting opportunity to provide new insights for optimizing chlamydia control. Basic scientists, clinical researchers, and epidemiologists will need to join forces in conducting coordinated research efforts to gain a better understanding of chlamydia immunobiology and to further the goal of preventing the adverse reproductive consequences of genital *C. trachomatis* infection.

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