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Human and Pathogen Factors Associated with *Chlamydia trachomatis*-Related Infertility in Women

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SUMMARY

Chlamydia trachomatis is the most common bacterial sexually transmitted pathogen worldwide. Infection can result in serious reproductive pathologies, including pelvic inflammatory disease, ectopic pregnancy, and infertility, in women. However, the processes that result in these reproductive pathologies have not been well defined. Here we review the evidence for the human disease burden of these chlamydial reproductive pathologies. We then review human-based evidence that links *Chlamydia* with reproductive pathologies in women. We present data supporting the idea that host, immunological, epidemiological, and pathogen factors may all contribute to the development of infertility. Specifically, we review the existing evidence that host and pathogen genotypes, host hormone status, age of sexual debut, sexual behavior, coinfections, and repeat infections are all likely to be contributory factors in development of infertility. Pathogen factors such as infectious burden, treatment failure, and tissue tropisms or ascension capacity are also potential contributory factors. We present four possible processes of pathology development and how these processes are supported by the published data. We highlight the limitations of the evidence and propose future studies that could improve our understanding of how chlamydial infertility in women occurs and possible future interventions to reduce this disease burden.

INTRODUCTION

Chlamydia trachomatis infection is one of the most common bacterial sexually transmitted infections (STIs) worldwide, with the WHO's most recent estimates indicating approximately 100 million new infections annually (1); in the United States alone, approximately 1.4 million *Chlamydia* infections occurred in 2013 (2). *C. trachomatis* is a Gram-negative, obligate intracellular bacterial pathogen with a unique biphasic developmental cycle (3). The organism has been characterized by using biology

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and genomics as a highly evolved or ancient pathogen with evidence of a reduced genome that is tailored for the obligate intracellular human niche (4). The infection can result in serious reproductive consequences, including pelvic inflammatory disease (PID), ectopic pregnancy, and infertility, in women. Genital *Chlamydia* infection is asymptomatic in over 70% of the cases, and as a result, few population-based prevalence or incidence estimates are available (5). Available population-based data from the United States, Australia, and the United Kingdom suggest that between 3 and 5% of people under 30 years of age will have a *Chlamydia* infection at any point in time (6–10). Estimates of the incidence of disease sequelae (specifically, PID, ectopic pregnancy, and infertility in women) are lacking, largely because there are very few natural-history studies of *Chlamydia* infections in humans. A systematic review attempting to establish the attributable risk of infertility among women following genital *Chlamydia* infection concluded that there is currently not sufficient evidence to accurately determine the population attributable risk (11). Surveillance of infertility on a population level is very limited, and thus it is difficult to ascertain whether an increased incidence of *Chlamydia* infection (or indeed the increased detection and treatment of infections) is associated with concurrent trends in infertility.

However, there is robust evidence that women who have experienced PID are more likely to develop infertility and robust evidence that *Chlamydia* infection causes PID. Specifically, a longitudinal cohort study was conducted by Weström et al. (12) to evaluate PID and fertility outcomes. The study followed 1,844 women with abnormal laparoscopic findings upon suspicion of acute PID (case subjects; not limited to *Chlamydia* infection) and 657 women who had normal findings (controls). Follow-up found that upon attempting to become pregnant, 16.5% of the case subjects and 2.7% of the controls failed to conceive (12). Further investigations demonstrated that 141 (10.8%) of the PID case subjects and none of the controls had developed tubal factor infertility (TFI) (12). In addition, the ectopic pregnancy rate was also higher in the PID case subjects, with 9.1% of the case subjects and 1.4% of the controls experiencing ectopic pregnancy (12). However, this study did not detail the infectious agent underlying the initial PID.

A randomized controlled trial to evaluate if chlamydial screening can prevent PID (prevention of pelvic infection trial) found that the risk of PID among women whose *Chlamydia* infections were left untreated was 9.5%, considerably higher than the 1.6% risk of PID among women whose infections were treated (13). Mathematical modeling suggested that PID can occur at any time point during the natural history of an infection; therefore, annual chlamydial screening programs may reduce the incidence of PID (14). A separate model concluded that annual screening could prevent 61% (95% credible interval, 55 to 67%) of *Chlamydia*-associated PID (15).

The risk of infertility due to *Chlamydia* infection is much lower than that of PID; for example, in Sweden, using a retrospective hospital record review process, it was found that women who had a positive *Chlamydia* test had an adjusted hazards ratio of 1.31 (95% confidence interval, 1.09 to 1.57; $P < 0.0001$) of having infertility compared to women who tested negative for *Chlamydia*, although there was no significant difference in ectopic pregnancy outcomes (16). The performance of the serological tests that are commonly used in conjunction with gynecological investigation to attribute infertility to *Chlamydia* is one of the limitations of the assessment of the burden of chlamydial infertility. However, a meta-analysis conducted in 2011 compared micro-immunofluorescence (MIF) tests and several enzyme-linked immunosorbent assays (ELISAs) and found that MIF tests had the highest accuracy (area under the curve) for a positive reaction in women with tubal pathology ($P < 0.001$; $P = 0.01$ for bilateral occlusion) (17). Regardless of test performance, numerous studies have identified a correlation of *Chlamydia* serology positivity (*Chlamydia* antibody testing) with diagnosed TFI (18–24). Analysis of these published data by using a model estimated that the proportion of tubal infertility caused by *Chlamydia* is 45% (credible interval, 28 to 62%) (25).

In spite of the considerable worldwide burden of chlamydial disease, the host and pathogen determinants that result in infertility are still not known. It is possible that identification of the risk factors for the development of serious sequelae could be used to implement additional targeted screening and treatment of at-risk women to reduce the burden of this disease. Furthermore, recent exciting advances have been made in the development of a chlamydial vaccine (26); however, it will be critical to establish that any vaccine will not have unwanted consequences for reproductive pathology in some women. Therefore, in order to inform future developments in the field, we have chosen to focus this review specifically on infertility in women. Infertility is defined as the inability to have a healthy live baby after 12 months of unprotected timed intercourse. The majority of the studies reviewed for this report state their definition of infertility as inability to achieve successful pregnancy after >12 months of unprotected sex. Some studies refer to subfertility, which is defined as reduced fertility with a prolonged time of unwanted nonconception, and we consider this compatible with infertility for the purpose of this review. PID and ectopic pregnancy are also reviewed here, with ectopic pregnancy defined as tubal development of the embryo. In order to identify what is known and what is not known about the host and pathogen factors that result in the development of infertility in women, we used PUBMED searches to identify and review the relevant literature to generate this review (focusing on direct human evidence). Our aim is to highlight the most solid or consistently identified factors that are known already and to identify the gaps in our knowledge of the risk factors that could predict or determine infertility outcomes in women that should be addressed in future studies to ensure optimal vaccine design and inform improved diagnostics.

CHLAMYDIAL FACTORS THAT MAY INFLUENCE THE DEVELOPMENT OF PATHOLOGY

Chlamydial Serovars Can Associate with Repeat Infection and Symptoms That May Also Relate to a Propensity To Cause Pathology and Infertility

Chlamydial serovars are assigned on the basis of the immune response to the major outer membrane protein and typically in more recent work by sequencing this locus (27). Serovars E and F are generally found to be predominant in most countries, including Australia, Netherlands, and Sweden (28–32). Using *omp1* PCR-based restriction fragment length polymorphism genotyping, Lan et al. (33) reported that in women <30 years old, serovars D (4/21 asymptomatic women) and I (4/21 asymptomatic women) were associated with asymptomatic infection, while serovar G was associated with symptomatic infection (4/30 symptomatic cases). Similarly, Gao et al. (30) also

reported the predominance of serovar G in symptomatic infection, particularly lower abdominal pain (18/33 patients), compared to asymptomatic patients ($P < 0.001$) among female sex workers and sexually transmitted disease (STD) patients in China. Of the asymptomatic patients, 47.5% (28/59) had serovar E (30). Verweij et al. reported that different serovars induced differential serological responses in 510 women with PCR-confirmed *C. trachomatis* infection (34). Their study showed that serovars D ($n = 45$) and E ($n = 217$) from serogroup B elicited the highest IgG responses (median IgG titer of 200), followed by serovar H ($n = 20$) (34). Women with persisting infections were found to have twice as may serovar E infections (67%, 8/12) than women who naturally cleared their infections (33% 3/9) in a longitudinal study of women with asymptomatic *C. trachomatis* infections; however, this was not a significant difference (35). However, several design flaws in the study done by Morré et al. in 2002 have been pointed out and therefore it may not be appropriate to draw conclusions from these data (e.g., see references 36 and 37). It is important to note that some infected women do not launch a detectable serological response against elementary bodies (EBs) from several serovars or antigens (independent of the infecting serovar), suggesting that not all infections result in a detectable humoral immune response (38). While not linked directly to infertility, the majority of the evidence from these studies supports the idea that different serovars do have different associations with symptoms, the immune response, reinfection, or infection duration, all of which may contribute to pathology development.

Chlamydial Genotypes Can Mediate Tissue Tropism and May Be a Factor in Pathology Development

There are several published reports of *C. trachomatis* genotypic differences associating with tissue tropism but no data yet aligning chlamydial genotypes with pathology development and infertility. The best-known genotype that mediates tissue tropism is polymorphism in the *trpAB* operon, which disables the synthesis of tryptophan from indole in *C. trachomatis* ocular strains but not in genital strains (39, 40). It is mentioned here because of the significance of this finding to the field even though this review is focused on infertility. There is also evidence of positively selected genotypes that align with tissue tropism (ocular, genital, mononuclear/invasive). For example, in a review of 59 *C. trachomatis* genome sequences, it was reported that the Tarp gene and the *pmp* genes have positively selected polymorphisms that cluster significantly with tissue niches (41). Polymorphisms in three distinct open reading frames have been found to associate with rectal but not cervical serovar G isolates, and two further open reading frame polymorphisms were found to associate with rectal and cervical tropism of serovars E, F, and J (42). These studies do not identify tissue pathology-associated polymorphisms; however, they do support the theoretical potential for chlamydial genotypes to exist that either increase the frequency of ascension or enhance upper reproductive tract survival or polymorphisms that alter the immune response. Determination of an association a chlamydial genotype with pathology development would be possible only by a longitudinal study of a large group of women throughout their reproductive years with continual sampling and analysis of infecting chlamydial strains.

IMMUNE RESPONSES ASSOCIATED WITH *C. TRACHOMATIS* IN WOMEN

Insight into the immunological factors that may be involved in *C. trachomatis* infertility in women has been obtained through various human studies, including local secretions (cervical and vaginal lavages), human tissues, and peripheral blood mononuclear cells (PBMC), as well as through *in vitro* cell culture models. However, while numerous studies are mentioned here (but not all, because of space limitations), many of these were on relatively small numbers of patients or immune factors, and in fact, there is very sparse evidence on the host immune response in women with chlamydial infertility. The innate response to infection is clearly critical for the infection outcome and, as outlined in the cellular paradigm process (discussed below), could be the major determinant of either a pathological result or resolution of the infection. A framework commonly used to categorize the adaptive immune response is the Th1-Th2 paradigm (reviewed in reference 43). This paradigm suggests that the immune response can polarize toward a cytotoxic response (Th1) or a humoral antibody-mediated response (Th2). The profiles of these responses include the Th1 subset of T helper (Th) lymphocytes and the production of cytokines such as interleukin-2 (IL-2), IL-6, tumor necrosis factor (TNF-α), and gamma interferon (IFN-γ) (44, 45), and Th2 responses involve IL-4, IL-5, IL-6, IL-10, and IL-13 (44). In addition to Th1-Th2, regulatory immune profiles typified by IL-17 or IL-23 have also recently been described (reviewed in reference 46). Here we summarize the existing evidence on the human immune response to *C. trachomatis* and how this evidence fits four different possible processes of infertility development (presented at the end of this review) by using these immune categories.

Immune Responses from Reproductive Sites and Tissues

Genital secretions have been used to reveal the immunological responses that occur at the site of infection, although it is not possible to link these with subsequent pathology. IFN-γ levels were found to be five times as high in the endocervical secretions of women with *C. trachomatis* infection detected by culture ($n = 47$) as in those of uninfected women ($n = 52$) (47). Analysis of immune factors by a multiplex immunoassay in cervical-vaginal lavage fluids of women with acute *C. trachomatis* infection ($n = 5$) attending an STD clinic compared to those of controls with no infections ($n = 13$) revealed significantly higher levels of IL-1β, lactoferrin, TNF-α, IL-8, vascular endothelial growth factor, granulocyte-colony stimulating factor (CSF), IL-10, IL-3, IL-7, IL-12, and IL-6 (48). In another study in India, IFN-γ levels were significantly higher only in women with recurrent *C. trachomatis* infections than in controls but not higher than in those with primary infections (49). IL-17 and IL-22 were five and three times as high in the cervical secretions of *C. trachomatis*-positive women ($n = 27$) as in negative controls ($n = 17$) (50). Titers of IgG and IgA antibodies to *C. trachomatis* EBs were higher in the cervical washes of *C. trachomatis*-positive fertile women during primary infection than during recurrent infection (49). However, the cervical titer of IgG to specific chlamydial antigens (cHSP60 and cHSP10) was higher in a recurrent infection than in a primary infection, which is what would be expected (49). Flow cytometric analysis of lymphocytes present at the cervixes of *C. trachomatis*-infected women identified the increased presence of a unique cell type, $\alpha4\beta7^+$ CLA^+ memory T cells (51). Stimulation of cervical monocytes

with chlamydial EBs showed increased expression of Toll-like receptor 2 (TLR2) and TLR4, and both receptors were also found to be expressed at higher levels in cervical cells from infected women than in those from uninfected women (52). The levels of IL-1β, IL-4, IL-5, IL-6, and IL-10 were reported to be significantly higher in enriched cervical T cells stimulated with chlamydial inclusion (Inc) membrane proteins from women with *C. trachomatis*-related infertility ($n = 18$) than in those from *C. trachomatis*-positive fertile women ($n = 14$) (53). While ectopic pregnancy is not only a *Chlamydia*-related pathology, a study by Balasubramaniam et al. that found that levels of the cytokines IL-8 and IL-6 were significantly upregulated in the fallopian tubes immediately at the location of implantation of ectopic pregnancy but not in the fallopian tubes of women undergoing benign hysterectomy (54). A very recent study found that differences in IFN-γ levels correlated with differences in the chlamydial cellular morphologies at the cervix when comparing two patients (55). In summary, the consistent findings of these studies are that IFN-γ, IL-6, IL-1, IL-8, and high antibody responses to some antigens are associated with chlamydial responses in the reproductive tracts of infertile women and *Chlamydia*-infected women (a notable caveat being that much of the evidence is from uncomplicated infections).

Examination of the effects of chlamydial infection in human tissue *ex vivo* has provided a controlled insight into the cellular responses that likely occur *in vivo* immediately upon primary infection. Through *ex vivo* fallopian tube studies, Hvid et al. (56) showed that the addition of IL-1 receptor antagonists blocked the IL-1 and IL-8 induced in response to *Chlamydia* and this prevented the pathology from chlamydial infection (56). This confirms that IL-1 and IL-8 are likely major factors in tubal pathology (56), supporting the cellular paradigm of pathology development and not supporting the hypersensitivity model. This study further implicates the innate immune response as a possible determinant of infection outcome.

Fallopian tube explants from women with ectopic pregnancy who were *C. trachomatis* seropositive were used to demonstrate that *C. trachomatis* induced higher expression of a prokineticin receptor via TLR2 binding (57). The prokineticin pathway impacts smooth muscle contraction and intrauterine implantation, as well as angiogenesis, which may imply a mechanism for *C. trachomatis* damage leading to ectopic pregnancy (57). T lymphocytes from endometrial and salpingeal tissues cultured *ex vivo* in the presence of *Chlamydia* or cHSP60 showed induction of lymphocyte proliferation and IFN-γ secretion from PID or ectopic pregnancy cases compared to cell lines (58). In the same patients, higher levels of IFN-γ mRNA expression and lower levels of IL-5 in fallopian tube and peritoneal cavity specimens from PID patients who had T cells proliferating in response to the organism *ex vivo* further implicated Th1 cytokine production in *C. trachomatis*-related pathology (58). However, it is important to note that these Th1 responses may be required to resolve the infection, and the clearance compared to the tissue pathology results from a Th1 profile immune response are not well understood. Primary endocervical cell cultures infected with *C. trachomatis* also demonstrated a proinflammatory cytokine response, with abundant production of IL-8, IL-1α, and TNF-α, all of which were maximally detected during live infection with active bacterial protein synthesis (59). These primary endocervical cultures indicate that the innate immune response to *Chlamydia* is typically proinflammatory, which is what may be required to successfully resolve the infection. Combined, these data predominantly support the cellular paradigm and ascending infection models of pathology and link to a Th1 profile of immunity. However, given that a cytotoxic response is needed to resolve an infection, differentiation between a successful Th1 profile or response associated with resolution of infection and one that results in tissue damage is not possible in the absence of longitudinal data.

Immune Responses in PBMC in Women

PBMC are often used to provide insight into how immune cell responses to *Chlamydia* relate to the patient infection or disease status. PBMC proliferation induced by purified *C. trachomatis* EBs showed prominent secretion of IFN-γ in women with genital infections (60). In a separate study, PBMC from healthy donors were incubated with *C. trachomatis* serovar K, leading to the finding that proinflammatory gene expression (measured by microarray and validated by quantitative reverse transcription-PCR) was sustained for up to 7 days, especially for IFN-γ, and IL-2 receptor γ (61). However, in one report, cHSP60-stimulated PBMC production of IFN-γ was lower in women with PID or repeat infections than in women with current infections (62, 63). Dendritic cells prepared from human PBMC were infected with *C. trachomatis* serovar E or L2, which induced the production of IL-1β, IL-6, IL-8, IL-12p70, IL-18, and TNF-α (64). Production of IL-12 and TNF-α by human PBMC-sourced dendritic cells infected with *C. trachomatis* L2 was reported (65). Hook et al. (66) showed that stimulation of PBMC with chlamydial EBs elicited IFN-γ production by natural killer cells. Cohen et al. (67) showed that IFN-γ production from PBMC stimulated with cHSP60 correlated with patients who were protected from acquisition of infection in a longitudinal study of 143 female patients (67). This study also showed that the PBMC production of IL-13 in response to chlamydial EBs also correlated with a reduced risk of infection (67). Our own research has demonstrated IL-6 production from endometrial and endocervical primary *ex vivo* cultures from live infections with *C. trachomatis* (68).

Immune Pathway Elucidated by *In Vitro* Culture Models

Epithelial cell cultures are most commonly used for *in vitro* growth and characterization of *C. trachomatis* infection (69). *C. trachomatis* infection of HeLa cells, SiHa human cervical carcinoma epithelial cells, and HEp2 human epithelial cells was used to demonstrate that IL-1α regulates IL-8 production in these cell lines during infection (70). Similarly, mRNA measurement of cytokine expression after *C. trachomatis* infection of HeLa cells, SiHa cervix squamous carcinoma cells, and HT-29 colon adenocarcinoma cells showed increased levels of IL-8, GROα, granulocyte-macrophage CSF, IL-6, and IL-1α (59). A HeLa–THP-1 coculture model attempting to recapitulate the *in vivo* cellular cross talk also identified IL-6 and IL-8 cytokine secretion that was sustained during *C. trachomatis* infection with IL-1β transiently detected (71). IL-6 and IL-8 were also detected by using cervical and epithelial cell infection models (59). A HeLa and THP-1 (mononuclear cell) coculture model found that there are distinct profiles of innate immune responses to *C. trachomatis* serovars E and L2. The results also showed a difference between the two serovars in the effectiveness with which monocyte-released innate cytokines (such as TNF-α) controlled the infection in an epithelial cell model (72). Using a similar coculture model, our team also demonstrated sus-

tained IL-6 production in response to *C. trachomatis* L2 infection (59). Examination of host and chlamydial gene expression by a high-throughput RNA sequencing method enabled the investigators to identify a fibrotic profile of gene expression being induced in infected HEP-2 cells (73), although it would be interesting to see the outcome of the same experiment with a reproductive tract cell line or primary *ex vivo* studies. The role of TNF-α in the inhibition of chlamydial development was evaluated by the addition of recombinant TNF-α to Hep2 cells prior to infection, which resulted in smaller inclusion bodies (74). Furthermore, addition of IFN-γ to the cell line showed that, in combination with TNF-α, chlamydial replication was inhibited (74). Lu et al. (75) showed that chlamydial infection of several epithelial cell lines resulted in the secretion of mature IL-18. IL-18 is known to potentiate the Th1 immune response and enhance the production of IFN-γ (75).

Recently, two teams demonstrated important links among apoptosis, cell survival, and carcinogenesis for *Chlamydia* (76, 77). They demonstrated that sustained p53 downregulation is the key event that prevents apoptosis in *Chlamydia*-infected cells and that this is actively mediated by *Chlamydia* via the p53-MDM2 axis and ubiquitination (76, 77). These important *in vitro* findings demonstrate that it is not just via innate immune and pathogen defense pathways that *Chlamydia* impacts the cell, but other central host cell pathways are also fundamentally required for the pathogen's survival. These studies support the ideas that IFN-γ has a central role in the immune response and quite probably infection clearance or prevention of infection and that, potentially, the level or combination of the immune response may modulate the resolution or pathological outcome of infection.

Conclusion

Overwhelmingly, the studies of *in vivo* responses, *ex vivo* cultures, and laboratory cell culture models all support a higher level or predominance of a Th1 or cytotoxic profile of immune response to chlamydial infection, with IFN-γ, IL-8, IL-1, and IL-6 findings featuring almost universally in the studies (although IFN-γ was also associated with protection against a repeat infection). However, it is not clear if the type or level of pathological response is moderated or different between women who develop pathology and those who resolve the infection without pathology. Longitudinal sampling and analysis of correlates of the local immune response, the PMBC response, and infection clearance relative to the initial infection burden with the development of upper reproductive tract pathology is one possible study design that could help to clarify the contribution of proinflammatory primary immunity.

HOST/HUMAN FACTORS ASSOCIATED WITH CHLAMYDIAL INFERTILITY IN WOMEN

Repeat Infection and Infertility

Repeat infection represents a substantial proportion of the chlamydial infections detected annually (reinfection review in reference 78). It is likely that these repeat infections are made up of reinfections, treatment failures, and persistent infections (79). Batteiger et al. studied a longitudinal cohort of 210 adolescent women (14 to 17 years old) in the United States and found that 121 experienced repeat infections (79). In a longitudinal cohort in Australia, 1,116 women (16 to 25 year old) were followed up and 14 reinfections were observed in a total of 81 women who were at risk of repeat infection (3 of these had two episodes of reinfection) (cumulative risk over 12 months of 20.3%; 95% confidence interval [CI], 13.2 to 37.6%) (32). Interestingly, in this study in Australia, the organism load was lower in reinfections than in prevalent infections detected at the study baseline (32) but there were no associations between patient characteristics and reinfection (32) (possibly because the study was underpowered to detect an effect). However, careful consideration of the study design is needed when evaluating repeat infection; the studies of Batteiger et al. and Walker et al. tested quarterly for *Chlamydia*, and PCR may detect residual DNA from dead organisms causing a false positive (i.e., increased frequency or timing of specimen collection can overestimate repeat infection). A study conducted in Vancouver, Canada, showed that repeat infections occurred in 8 of 42 patients in a longitudinal study (both genders), indicating a cumulative incidence of 29% (95% CI, 12 to 46%), although the study was confounded by the immediate retesting of some patients (80). A study in the United Kingdom showed that the reinfection rate was 29.9 (range, 19.7 to 45.4) per 100 person years in a general practice clinic setting (81). Interestingly, it appears that women who resolve their infections spontaneously between diagnosis and returning for treatment are more protected from a repeat infection (82). As we would expect, infection and repeat infection are significantly associated with sexual behavior risk factors such as new partners or failure to use condoms (32). These repeat infection rates appear to be higher than the reported infertility outcome rates of women who had a positive *Chlamydia* test in the retrospective cohort study of Low et al. (16), suggesting that repeat infection is not the sole determinant of infertility or pathology. However, there is evidence that repeat infection increases the risk of developing infertility. A retrospective cohort study by Hillis and coworkers in Wisconsin examined the risk of hospitalization for ectopic pregnancy or PID in women who had one or more chlamydial infections (n = 11,000). They identified higher risks of ectopic pregnancy in women who had two (odds ratio [OR], 2.1; 95% CI, 1.3 to 3.4) or three or more (OR, 4.5; 95% CI, 1.8 to 5.3) chlamydial infections (83). The PID risk was also higher for women who had two (OR, 4.0; 95% CI, 1.6 to 9.9) or three or more (OR, 6.4; 95% CI, 2.2 to 18.4) chlamydial infections (83). Therefore, repeat infection is likely to be a significant contributing factor in at least some cases of chlamydial infertility.

Treatment failures induced by antibiotics have also been proposed to contribute to repeat infection rates (reviewed in reference 84). Batteiger et al. (79) showed that repeat infections in 13.7% of their patients were due to possible or probable treatment failures. Additionally, *in vitro* studies have shown that azithromycin induces chlamydial persistence or heterotrophic resistance (84–87). Therefore, treatment failure or perhaps indirect failure via persistence could be a contributing factor in the development of infertility in some women via all of the proposed mechanisms.

The rise of repeat infections detected over time has resulted in the proposal that increased public health investment in chlamydial screening and treatment is preventing the development of natural immunity to the infection (arrested-immunity hypothesis) (88, 89). Furthermore, the authors propose that these repeat infections may be the source of pathological sequelae that may not occur in women who have protective immunity from a naturally resolved infection (88, 89).

Do Coinfections or the Microbiome Play a Role in Chlamydial Pathology?

Coinfections with other STIs are common; for example, 46% of female patients positive for *Neisseria gonorrhoeae* were also positive for *Chlamydia* in a family planning clinic in the United States (90). However, a serology study in Zimbabwe demonstrated that women with antibodies to either *C. trachomatis* or *N. gonorrhoeae* were significantly more at risk of having PID, ectopic pregnancy, or abnormal fallopian tubes than were women with antibodies to both pathogens (91), suggesting that infection with both pathogens is not necessary for reproductive pathology. But this study needs to be interpreted with caution, as the antibody tests for the gonococcus are not sufficiently sensitive. Numerous studies have implicated bacterial vaginosis in chlamydial infection (92–96). Patients with bacterial vaginosis who had recent contact with a male with chlamydial urethritis had an OR of 3.4 (95% CI, 1.5 to 7.8) to test positive for chlamydial infection. The presence of H_2O_2-producing lactobacilli in the vagina were protective against the acquisition of infection (OR, 0.4; 95% CI, 0.2 to 0.8) (93). Recently an *in vitro* model was used to demonstrate that it is actually the acid or pH-lowering impact of lactobacilli that confers antichlamydial activity (97). It has been hypothesized that indole-producing organisms that increase in the microbiome during or subsequent to bacterial vaginosis enable *C. trachomatis* to synthesize tryptophan from indole (98), consequently evading the activity of IFN-γ at the infected site, and thus may facilitate infection (40, 98). Bacterial vaginosis during *C. trachomatis* or *N. gonorrhoeae* infection was recently found to be associated with a risk of PID, although the authors conclude that it is difficult to determine if the bacteria underlying the vaginosis facilitated ascension and PID caused by the STIs or, alternatively, if the bacteria responsible for vaginosis cause the PID (99). Clearly, there is an increased chlamydial infection risk associated with coinfection or bacterial vaginosis and also evidence of an association with PID, supporting the idea that coinfections could therefore result in an increased likelihood of development of infertility.

Sexual Behavior and Age of Sexual Contact

Age is a risk factor in the likelihood of contracting a chlamydial infection, with younger people at higher risk (10, 100). Cross-national studies from 1999 to 2008 in Denmark, Australia, and Sweden showed that increasing PID rates were associated with increasing age (15 to 19 year olds generally had the lowest number of cases per 100,000 women, while 30 to 34 or 35 to 39 year olds had the highest number of cases per 100,000 women), with the exception of New Zealand, where the rates were higher in women 15 to 19 years old (101). The highest incidence of *C. trachomatis* infection is in women 16 to 24 years old (32). Consistent with these studies, being under 20 years old and presenting with cervical symptoms were risk factors for endocervical chlamydial infection in women, and the organism burden was also higher in younger women when measured by viable culture counts (102).

Sexual behavior (new partners or a higher number of partners) is often significantly associated with an increased risk of *C. trachomatis* infection (7, 103). As shown in the study by Skjeldestad et al. (104), where there was a 37% probability of a woman acquiring *C. trachomatis* infection if she has had three or more partners within 42 months. A younger age at first coitus, a higher number of sexual partners, and a self-reported history of medically diagnosed STI were all significantly associated with tubal infertility, in contrast to fertile controls ($P < 0.01$) (105). However, as these are also known to be significant factors in the increased likelihood of acquisition of chlamydial infection, it is not certain if they directly influence the likelihood of pathology outcomes from the infection.

Hormonal Status at Time of Infection

Oral contraceptive use was found to be a risk factor for *C. trachomatis* infection ($P = 0.006$), and the organism burden was found to be higher in oral contraceptive users ($P < 0.001$) (102). Prospective recruitment of young female patients (≤17 years of age) attending genitourinary medicine clinics in the United Kingdom found that those who had a *Chlamydia* infection were also more likely to have elevated progesterone concentrations ($P = 0.05$) (106). In contrast, oral contraceptive use has been linked to protection from subsequent infertility or conception problems by two different studies (12, 107). However, it would be extremely difficult to link hormone status at time of infection with subsequent pathological outcomes such as infertility, even in a longitudinal study, given the high numbers of oral contraceptive users and the independent importance of these factors in the risk of acquiring an infection.

Human Genotypic Factors Associated with *C. trachomatis* Infertility in Women

The development of *C. trachomatis*-related infertility in only some women may be a consequence of a genotypic predisposition (reviewed comprehensively in reference 108). These studies are described below; however, for the sake of brevity, we have documented the sample sizes, statistics, and details of the alleles in Table 1. Note that since a comprehensive review of this topic (mentioned above) has recently been published, this section is brief (108).

TLRs recognize and bind to antigens of a pathogen and signal to upregulate an immune response. Ten TLRs have been identified in the human body, and TLR1 to TLR4 are expressed in the female genital tract (109). TLR2 and TLR4 are also expressed on various cells (110). In a prospective genotypic study with female patients attending a sexual health clinic and a fertility clinic in Amsterdam, Netherlands, Karimi et al. (111) demonstrated a possible protective role for a TLR2 haplotype. The haplotype, consisting of two distinct single nucleotide polymorphisms (SNPs), was found to be present in a significantly higher proportion of women with a history of *Chlamydia* infection who did not have tubal pathology and in sexual health clinic attendees and was present in a higher proportion of women who were asymptomatic (111). In the PEACH PID study TLR1 and TLR4 polymorphisms were found to be significantly associated with chlamydial PID (112), while SNPs from TLR2 (SNPs different from those included in the study of Karimi et al.), TLR6, Myd88, and TIRAP were not significantly associated with chlamydial PID (112).

den Hartog et al. (113) found that the risk of tubal pathology was higher in *C. trachomatis* IgG-positive women with multiple TLR polymorphisms. That study also showed a trend toward the association of various individual polymorphisms in TLR9 and TLR4 with an increased risk of tubal pathology in *C. trachomatis* IgG-positive women (113). However, the small sample size in that study makes it difficult to draw a correlation between the polymorphisms and the level of risk of female infertility.

NLRP3, a component of the inflammasome that activates a pro-

TABLE 1 Summary of genotypes that have been significantly associated with chlamydial infection or pathology

Locus	Study parameters	Significance	Reference
TLR1 (rs5743618), *TT*	Prospective recruitment in PEACH PID study; 111 women with PID confirmed *C. trachomatis* negative by PCR, 94 women with PID confirmed *C. trachomatis* positive by PCR	*TT* genotype, increased risk of PID with chlamydial infection (adjusted OR, 2.8; 95% CI, 1.3–6.2; $P = 0.008$)	112
TLR2-16934 (T>A [rs4696480], *+2477* G>A [rs5743708])	Fertility clinic, 17 *C. trachomatis* IgG-negative women with tubal pathology, 13 *C. trachomatis*-positive women without tubal pathology, 26 *C. trachomatis* IgG-positive women with tubal pathology (*C. trachomatis* status: MIF test titer, >1:32); sexual health clinic, 147 *C. trachomatis* IgG-positive women, 81 *C. trachomatis* IgG-positive asymptomatic women, 66 *C. trachomatis* IgG-positive symptomatic women, 321 *C. trachomatis* IgG-negative women	Individual SNPs not significantly associated with infection, risk, symptom, or infertility; haplotype, −16934A with +2477G; higher in women with *C. trachomatis* but not tubal pathology ($P = 0.015$; OR, 0.28; 95% CI, 0.1–0.75); decreased proportion when women ranked from asymptomatic to symptomatic infections to *C. trachomatis* positive and tubal infertility	111
TLR4 (rs1927911 *CC* allele)	Prospective recruitment in PEACH PID study, 111 *C. trachomatis*-negative (PCR) women with PID, 94 women with PID *C. trachomatis* positive by PCR	*CC* allele significantly associated with *Chlamydia* positivity in PID participants ($P = 0.002$)	112
CD14 (−260C>T)	Whole blood from 44 healthy females, distribution of TNF-α allele among male and female participants in Taiwan (26 with *TT*, 36 with *C. trachomatis*, 22 with *CC*)	*TT* allele associated with significantly higher TNF-α production when whole blood stimulated with *C. trachomatis* ($P < 0.05$; relatively minor differences in actual concn)	124
	Dutch women of Caucasian origin *C. trachomatis* positive by MIF test with titer of ≥1:32; STD clinic, 135 *C. trachomatis* IgG positive, 49 *C. trachomatis* IgG negative, $n = 49$; subfertility clinic, 41 *C. trachomatis* IgG positive, 212 *C. trachomatis* IgG negative, 28 *C. trachomatis* IgG positive with tubal pathology, 22 *C. trachomatis* IgG negative with tubal pathology, 170 healthy controls	No significant difference in distribution of CD14 polymorphism in STD or infertility cohort	153
NLRP3 (AG or AA at SNP [rs12065526])	Sexual health clinic; 99 *C. trachomatis*-positive women, 44 *C. trachomatis*-positive symptomatic women, 219 *C. trachomatis*-negative women,, 75 *C. trachomatis*-negative symptomatic women, 93 healthy fertile controls	Those with AG or AA at SNP rs12065526 were at significant risk of developing abdominal pain when *C. trachomatis* infected ($P = 0.047$; OR 2.9; 95% CI, 1.8–8.0; not significantly associated with infection risk or tubal pathology)	114
MBL (high-MBL2-producing genotypes [*HYA/HYA*, *HYA/LYA*])	76 *C. trachomatis* IgG-positive women with TFI (total $n = 157$), 71 *C. trachomatis* IgG-positive women without TFI (total $n = 398$)	High-MBL2-producing genotypes *HYA/HYA* ($P = 0.054$; OR 3.72; 95% CI, = 1.07–12.78) and *HYA/LYA* ($P = 0.019$; OR = 0.28; 95% CI, = 0.11–0.75) higher in *C. trachomatis*-positive women than in *C. trachomatis*-positive women with TFI; MBL2 genotypes *LXA/LXA* higher in *C. trachomatis*-positive women with TFI than in *C. trachomatis*-positive women without TFI ($P = 0.025$; OR 2.34; 95% CI, 1.17–4.69)	110
HLA class II allele (*DRB1*1503*, *DRB5*0101*)	Infertility clinic in Kenya; 33 *C. trachomatis* IgG-positive women with TFI (MIF titers, ≥1:16), 37 *C. trachomatis* IgG-negative women with TFI	*DRB1*1503* detected in 7 women with TFI who were *C. trachomatis* negative and absent in *C. trachomatis*-positive women with TFI ($P = 0.01$; OR 0.05; 95% CI, = 0–0.7); *DRB5*0101* more common in *C. trachomatis*-seronegative women with TFI than in *C. trachomatis*-seropositive women with TFI ($P = 0.05$; OR = 0.2; 95% CI, 0.02–1)	116

(Continued on following page)

TABLE 1 (Continued)

Locus	Study parameters	Significance	Reference
Cytokines IL-10 (1083AA), TNF-α (−308A), IL-12B (rs3212227, AC), IL-10 1082A	114 infertile women at Helsinki infertility clinic, 176 fertile controls, 96 *C. trachomatis* IgG-positive women with TFI (MEDAC ELISA kits)	IL-10 increased risk of tubal damage in *C. trachomatis*-positive women (OR, 7.3; 95% CI, 1.3–4.2); TNF-α increased risk of tubal damage in *C. trachomatis*-positive women (OR, 4.0; 95% CI, 1.0–16)	120
	100 women with TFI, 125 pregnant fertile controls, 94 *C. trachomatis* IgG-positive women with TFI, 69 *C. trachomatis* IgG-negative women with TFI, 90 *C. trachomatis* cHSP60 IgG-positive women with TFI, 73 *C. trachomatis* cHSP60 IgG-negative women with TFI	IL-12B AC heterozygote increased risk of chlamydial TFI (OR, 2.44; 95% CI, 1.23–4.87)	121
	47 women with TFI at IVF clinic in Helsinki, 23 women with *C. trachomatis* IgG antibodies	IL-10 1082A allele frequency higher in *C. trachomatis*-positive TFI cases than in *C. trachomatis*-negative TFI cases (0.72 vs 0.46; $P = 0.01$)	118
MICA[a]	63 women with TFI at infertility clinic in China, 151 women without TFI, 42 *C. trachomatis* IgG-positive (determined by commercial immunoassay kits) women with TFI, 59 *C. trachomatis* IgG-positive women without TFI	*MICA*008* higher in infertile patients without *C. trachomatis* infection than in infertile women with *C. trachomatis* infection ($P = 0.00367$; OR, 2.14; 95% CI, 1.4–3.28); *MICA* allele protects against *C. trachomatis* tubal infertility	154

[a] *MICA*, major histocompatibility complex class I chain-related A.

inflammatory response, has been found to have polymorphisms reported to result in altered secretion of IL-1β (a proinflammatory cytokine) (114). Several NLRP3 polymorphisms were tested in women prospectively recruited at a sexual health clinic and fertility clinics in Amsterdam; women heterozygous or homozygous with the one of the NLRP3 polymorphisms were at significant risk of developing abdominal pain during *C. trachomatis* infection (114).

Mannose-binding lectin (MBL) is a proinflammatory protein that activates complement and is locally synthesized in the vagina (115). MBL and MBL promoter polymorphisms have been previously demonstrated to alter the levels of MBL produced (115). It was found that women with TFI who were *Chlamydia* seropositive more frequently had the low-MBL-producing genotypes than did healthy controls (115). However, this was not the case in an ectopic pregnancy group, who were less likely to have the MBL-deficient genotypes (115). The latter finding sheds some doubt on the role of MBL genotypes, given that it seems likely that the pathology associated with chlamydial ectopic pregnancy is similar to that resulting in chlamydial tubal infertility.

Human leukocyte antigen (HLA) class II molecules (DR, DQ, and DP) present peptides to CD4 T cells and induce adaptive immunity. DQ polymorphisms were more frequent in women with tubal infertility who were also *C. trachomatis* seropositive (116), while another polymorphism was negatively associated with *C. trachomatis* tubal infertility (116). Wang et al. (117) showed that in adolescent females with recurrent chlamydial infections, certain HLA polymorphisms were significantly associated with recurrent infections. A recent study attempted to link different immune genotypes to immunological outcomes by testing a functional role for HLA DQ alleles combined with an IL-10 allele both for frequency in a TFI (*Chlamydia*-positive) cohort compared to controls and for a lymphocyte proliferative response to cHSP60 (118). The HLA alleles and IL-10 allele were more frequently identified in the chlamydial tubal factor cohort than in the controls; however, the lymphocyte proliferation in response to cHSP60 was not significantly different in relation to the alleles (118). However, in a different study, distinct IL-10 and IFN-γ polymorphisms were found to have a significant impact on lymphocyte proliferation in response to chlamydial antigens (119).

A range of cytokine polymorphisms have been shown to significantly associate with women with *C. trachomatis* tubal infertility, including IL-10 (120), TNF-α (120), and IL-12 (121). Polymorphisms in IL-1β and receptor genes were not associated with *C. trachomatis*-related tubal pathology (122). Adolescent females with recurrent chlamydial infections had a lower frequency of three IL-10 promoter polymorphisms (117). Eng et al. (123) demonstrated that a CD14 allele (TLR-4 coreceptor) was associated with increased TNF-α production when whole blood was stimulated with *C. trachomatis* and *C. pneumoniae* (124).

While none of these studies were able to account for all chlamydial tubal infertility cases, they are a convincing body of evidence that the human genotype may be a factor in pathology development. It is interesting that most of the studies done to date have focused on immune factors, yet as an obligate intracellular pathogen, *Chlamydia* has key nutritional, inclusion vacuole, and structural requirements, and therefore, the ability to ascend and cause pathology may, in fact, be independently determined by an as-yet-unknown host genotype that could relate to these nutritional and structural requirements.

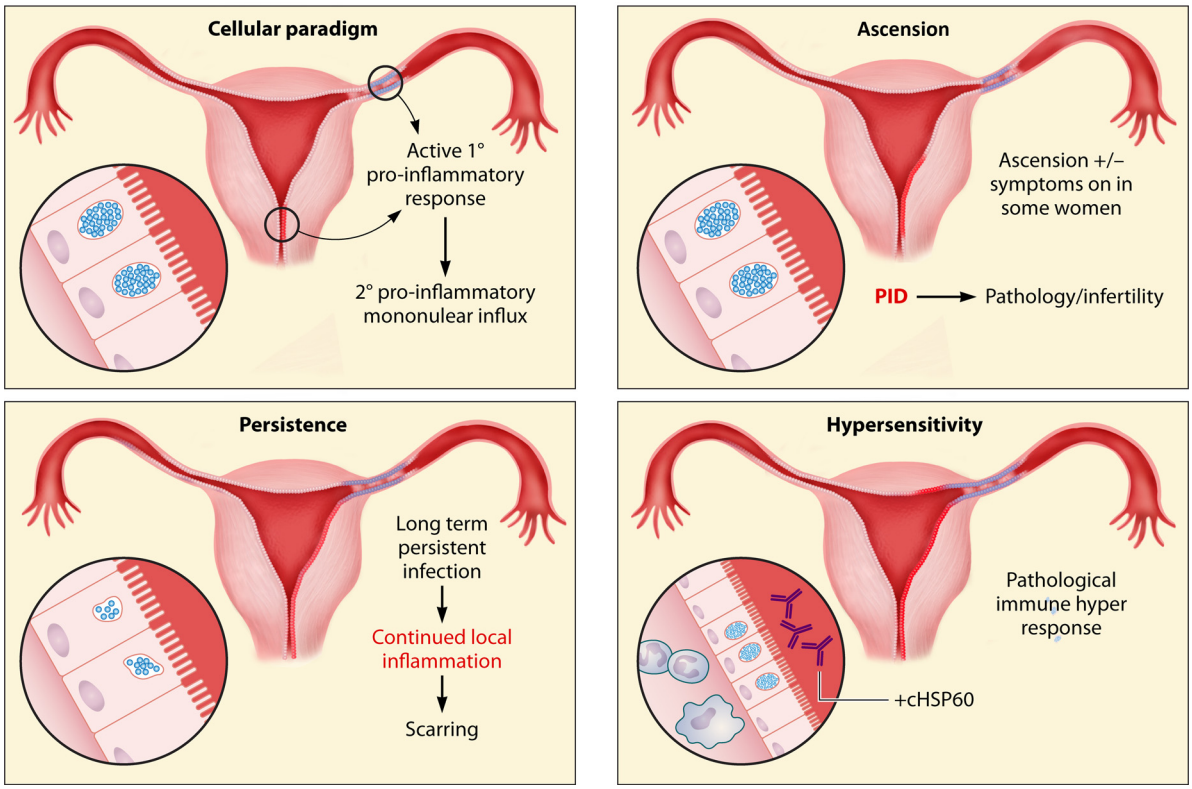

FIG 1 The four major models of development of pathology associated with chlamydial infertility in women. Shown are the four models summarized here that are supported within the field by evidence from human studies.

PROPOSED MECHANISMS OF THE DEVELOPMENT OF INFERTILITY FOLLOWING *C. TRACHOMATIS* INFECTION IN WOMEN

We describe what we consider to be four mechanisms or processes of host-pathogen interaction that are commonly referred to in the field to explain the process that underlies the development of infertility following *Chlamydia* infection. The processes are not exclusive of each other, and there is evidence for and against a role for each process. We will briefly summarize these processes so that the host and pathogen data we have reviewed can be considered in the context of each process or mechanism (Fig. 1).

Ascension

It is often suggested or assumed that infection ascends to the upper reproductive tract in only some women, and if it is unchecked, once it is in the upper reproductive tract, it results in the development of tubal pathology. There is considerable direct evidence that a chlamydial infection can ascend beyond the cervix; however, it is not known if ascension occurs without development of tubal pathology. Evidence of ascension includes the detection of *Chlamydia* DNA in salpingectomy specimens from one woman with ectopic pregnancy and in several endometrial and cervix specimens from women with subsequent ectopic pregnancy although not in their ectopic salpingectomy specimens (125). *C. trachomatis* DNA was detected in tubal specimens from women with ectopic pregnancy in Myanmar (126). *C. trachomatis* DNA was detected in the endometrium, fallopian tubes, and ovaries of as many as 56% of women with ectopic pregnancy or TFI (127). The presence of *C. trachomatis* organisms was associated with endometritis (plasma cell influx in the endometrium) (128, 129). A small study in the United Kingdom identified the presence of *C. trachomatis* in the upper genital tracts of 4 of 10 women, all of whom had no symptoms (130).

It seems possible or even likely that ascension is independently moderated by numerous other factors that could explain the low frequency of pathology development, supporting the idea that it occurs only in some women. These moderating factors could include host factors such as cycle stage or hormone status at the time of infection, as chlamydial or gonococcal endometritis was found to be more likely to be detected in women in the proliferative phase (94). Equally important moderating factors could include immune status, genotypic factors, other coinfections, or reproductive tract microbiome composition. Unfortunately, there is no published evidence on these factors or direct evidence that ascension does or does not occur. Furthermore, pathogen factors could also moderate the likelihood of ascension, such as the infectious burden (although it has not been possible to significantly associate the chlamydial serovar alone with pathogenic potential or clinical manifestations [131]). Certainly, it seems widely agreed upon that ascension and presence of the pathogen, rather than an indirect process such as molecular mimicry, are involved in pathology development. Yet it seems that ascension of the infection may also occur without pathology, so ascension may not be the primary determinant of pathology.

Persistence

Chlamydia persistence, or the presence of viable but nonculturable chlamydial organisms (visualized as aberrant forms), de-

velops in response to various conditions and can remain dormant for considerable lengths of time (reviewed in reference 132). In this review, we refer to *in vivo* correlates of the *in vitro* chlamydial persistent forms that remain indefinitely associated with cells and dormant and able to be reactivated. This is not the same as infections with other microbes that are referred to as "persistent" because the infection failed to respond to antimicrobial treatment. Persistence *in vivo* has been proposed as a model where this continued presence of the organism in aberrant body forms may drive a long-term pathological immune response resulting in tissue damage to the fallopian tube (certainly, the high expression of the dominant antigen cHSP60 has been detected in laboratory persistence models) (reviewed in reference 133). There are studies that have presented evidence of *C. trachomatis* DNA or antigens being present in the tubal material of women with tubal infertility or ectopic pregnancy or salpingitis, supporting the idea that organism persistence in some form occurs *in vivo* (127, 134–136). Recently, morphologies consistent with laboratory models of persistence were observed in endocervix samples associated with IFN-γ, further supporting the likely *in vivo* relevance of laboratory models of persistence (55). It is difficult to determine if chlamydial persistence leading to pathology has always occurred. Persistent infection may resolve at any time after pathology develops and prior to detection, and given the difficulties of specimen collection from the upper genital tract, the evidence is reasonably compelling that chlamydial persistence (consistent with either the lab models or a distinct *in vivo* form) is a relevant factor in pathology.

It is important to note a recent review that suggested that some forms of persistence may be "colonization" and in fact not associated with "pathogenesis" and disease (137). Our suggestion is that perhaps the two are distinct and that pathology-associated persistence (given the evidence reviewed here) and separate cases of nondamaging colonization occur. Specifically, the evidence that (i) chlamydial persistence is induced by a range of relevant conditions *in vitro* (reviewed in references 132 and 133) and (ii) persistence-like morphology and/or chlamydial antigen/DNA detection in the absence of active infection has been observed in clinical samples leads us to feel confident enough to suggest that persistence may be a relevant physiological status *in vivo*, particularly in the case of ascension to the upper reproductive tract tissues, and therefore likely a factor in a pathological outcome of infection.

Cellular Paradigm

The cellular paradigm mechanism proposes that the *Chlamydia*-infected epithelium immediately responds to the presence of the *Chlamydia* or specific antigens from *Chlamydia* in a proinflammatory manner, typified by proinflammatory cytokines and growth factors, which in turn induces a proinflammatory secondary immune cell activation and migration to form a local lymphoid follicle, resulting in cell damage and fibrosis or scarring (comprehensively presented and reviewed in reference 138). It is important to note that a proinflammatory response is also needed for clearance of the infection, given that the organisms is an intracellular pathogen (139). This mechanism is widely supported by the published evidence of human epithelial and immune cell responses to *Chlamydia* and a primary tissue *ex vivo* model that is discussed in more detail below. This mechanism also presumes that chlamydial ascension is associated with pathology and also mutually allows the persistence of *Chlamydia* in the upper reproductive tract (138). A pathological adaptive memory response proposed as the secondary component of this mechanism is also supported by the existing data that repeat infections increase the likelihood of pathological outcomes in women (83) and by numerous immunological results (as discussed here). However, it is also important to note that repeat infections would induce new acute or innate inflammatory responses that could drive the pathology independent of the secondary phase of the cellular paradigm mechanism.

cHSP60-Induced Delayed Hypersensitivity

There has been considerable focus on a mechanism that involves delayed hypersensitivity and/or molecular mimicry in response to specific chlamydial antigens (reviewed in reference 140). This has largely resulted from a body of literature demonstrating high-titer human antibody responses to cHSP60 in patients with tubal pathology and additionally that, in several animal models, tissue pathology developed after repeated inoculations with cHSP60 protein (reviewed in reference 138). However, this mechanism is a subject of considerable controversy because of inconsistencies between the different published findings, i.e., problems with protein preparations that involved a hypersensitive detergent being used in some animal models and cross-reactivity or poor specificity of some of the antibody tests (141–147). On the other hand, there is no doubt that cHSP60 is a predominant chlamydial antigen that does frequently elicit an immune response. Furthermore, in human trachoma cases, it is frequently possible to observe ongoing follicles present and disease pathology in the absence of PCR-detectable *Chlamydia*, suggesting that there is continuing immune activity in the absence of the organism during pathology development (148).

Interestingly, one group has proposed that cHSP60 antibodies detected in the follicular fluid of female *in vitro* fertilization (IVF) patients may induce early destruction of the embryo by cross-reacting to embryo human HSP60 and result in lower transfer success (149). They observed 74.1% cHSP60 seropositivity in women without embryo implantation ($n = 47$) and 47.9% seropositivity in women with successful embryo implantation ($n = 91$) ($P = 0.0004$). However, this observation directly contradicts a finding of a separate and much larger study ($n = 1,279$) that cumulative IVF cycle pregnancy rates are the same for women who are *Chlamydia* seropositive and those who are seronegative (150). A study in France also contradicted a possible role for antichlamydial immunity in embryo implantation failure, as it was found that the semen characteristics and IVF pregnancy rate of men and women with either PCR or serological evidence of *Chlamydia* ($n = 52$) were not different from those of controls who were negative by chlamydial testing ($n = 119$) (151).

CONCLUSIONS

A better understanding of the pathogenesis of *Chlamydia*-associated infertility and the risk factors associated with *C. trachomatis* infertility would enable specific screening for at-risk women and inform the development of a vaccine designed to prevent the progress of infection to infertility or pathology. While there are several convincing mechanisms that have been presented to explain how the disease pathology occurs, each with some supporting evidence from the human data reviewed here, the actual pro-

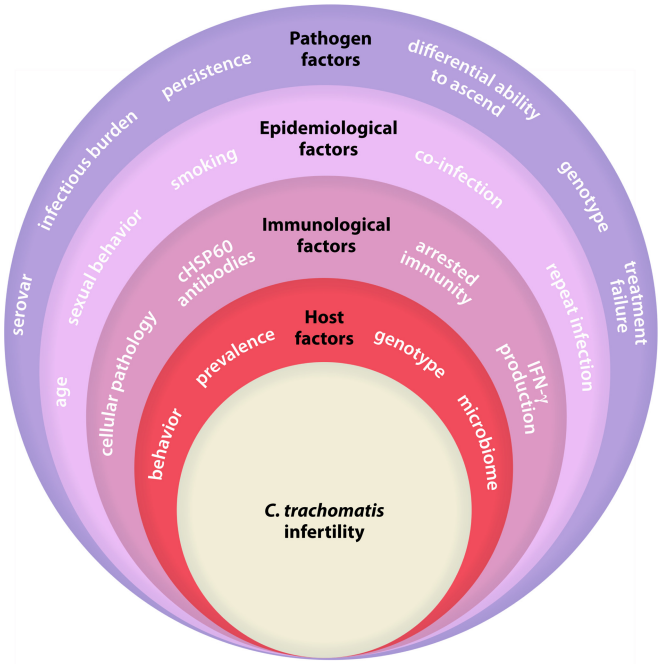

FIG 2 Summary of host and pathogen factors for which there is some evidence of contribution to the development of chlamydial infertility in women.

cess or host and pathogen factors that result in infertility remain uncertain and require further investigation. It is clear that a proinflammatory response is largely elicited in response to chlamydial infection. Whether this response or the extent of this response is the primary determinant of pathology remains unclear. Certainly, it is essential to have at least IFN-γ (considered a proinflammatory cytokine) and other proinflammatory activity for successful resolution of the infection. Furthermore, while there are tantalizing associations, there are not yet definitive correlates of the contributions of many host and pathogen factors to pathology risk, including human genetic polymorphisms, *C. trachomatis* serovar- and genotype-specific distinctions, coinfection or microbiome parameters, and risk behaviors like repeat infections or age at sexual debut (summarized in Fig. 2). A comprehensive and extended longitudinal analysis of each of these factors in a study of a very large cohort of women prior to any infection from the early teen years until postpregnancy age (with adequate statistical power) would be the most robust way to monitor disease factors and pathology outcomes for women (although such a study many not be feasible). Certainly, there is sufficient evidence that primary chlamydial screening is important to prevent PID and TFI. Identification of the major contributing factors to disease pathology would better inform the design of future vaccines and would also enable the design of molecular diagnostics for early detection of women at risk of pathology development.

In the absence of such comprehensive knowledge and subsequent preventive interventions (enhanced diagnosis or vaccine), there is a real opportunity to alleviate some of the risks and costs associated with the pathological outcome of infection by improving guidelines for screening, as recently proposed in the United Kingdom (152). In addition to this, we propose that the improved use of a serological diagnosis of pathology risk, expediting infertility diagnosis for women and also as a tool to predict the risk of ectopic pregnancy or PID in women (20, 150) who have had chlamydial infections, should also be rigorously evaluated and considered for routine implementation in both fertility and general practice clinic settings.

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