Pathogenesis of Genital Tract Disease Due to *Chlamydia trachomatis*

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Although the pathologic consequences of *C. trachomatis* genital infection are well-established, the mechanism(s) that result in chlamydia-induced tissue damage are not fully understood. We reviewed in vitro, animal, and human data related to the pathogenesis of chlamydial disease to better understand how reproductive sequelae result from *C. trachomatis* infection. Abundant in vitro data suggest that the inflammatory response to chlamydiae is initiated and sustained by actively infected nonimmune host epithelial cells. The mouse model indicates a critical role for chlamydia activation of the innate immune receptor, Toll-like receptor 2, and subsequent inflammatory cell influx and activation, which contributes to the development of chronic genital tract tissue damage. Data from recent vaccine studies in the murine model and from human immunoepidemiologic studies support a role for chlamydia-specific CD4 Th1-interferon-γ-producing cells in protection from infection and disease. However, limited evidence obtained using animal models of repeated infection indicates that, although the adaptive T cell response is a key mechanism involved in controlling or eliminating infection, it may have a double-edged nature and contribute to tissue damage. Important immunologic questions include whether anamnestic CD4 T cell responses drive disease rather than protect against disease and the role of specific immune cells and inflammatory mediators in the induction of tissue damage with primary and repeated infections. Continued study of the complex molecular and cellular interactions between chlamydiae and their host and large-scale prospective immunoepidemiologic and immunopathologic studies are needed to address gaps in our understanding of pathogenesis that thwart development of optimally effective control programs, including vaccine development.

Sexually transmitted *Chlamydia trachomatis* infection is a widespread public health concern because of its prevalence and potentially devastating reproductive consequences, including pelvic inflammatory disease (PID), infertility, and ectopic pregnancy. Although the pathologic consequences of infection are well estab-

lished, the mechanism(s) of chlamydia-induced tissue damage are not fully understood. Histological examination of tissue samples from women with PID caused by *C. trachomatis* revealed neutrophils in endometrial surface epithelium and in gland lumens, dense subepithelial stromal lymphocytic infiltration, stromal plasma cells, and germinal centers containing transformed lymphocytes [1]. The prominence of both neutrophils and chronic inflammatory cells in infected human female genital tract tissue samples does not assist in the determination of specific responses responsible for disease sequelae.

Because of the inherent difficulties in acquiring human tissue samples for study, researchers have taken advantage of multiple animal models of chlamydial infection to examine the nature and timing of the inflammatory response that occurs in the female genital tract after in vivo infection. Mouse and guinea pig models show that the response to primary chlamydial in-

fection occurs within 1–2 days of infection and is characterized by mucosal infiltration with neutrophils and modest numbers of monocytes [2–5]. Neutrophils are recruited in large numbers to the site of infection and are capable of killing accessible elementary bodies. Later, T cells accumulate at the site of chlamydial infection and play a critical role in controlling the infection (Figure 1) [6–8]. A consistent observation in the murine model is that tubal dilatation is a frequent end result of primary infection. Thus, the inflammatory process resulting from a single chlamydial insult may be sufficient to induce tubal damage and infertility. In contrast, in female guinea pigs, the host response to primary infection results in long-term tissue damage in a minority of infected animals [5, 9]. Analogous information on the proportion of women developing tubal damage after chlamydial infection is not available, but most infected women do not appear to develop clinical complications [10, 11]. Taking into consideration reported complication rates together with the high prevalence of genital tract *C. trachomatis* infection in women [12], it appears that the guinea pig model may more closely approximate human disease. In female animals and women, the multidimensional set of immune events that occurs at the time of infection ultimately results in an acquired immune response. Unfortunately, the adaptive response induced by infection is not effective in preventing reinfection, and the host's oviducts remain vulnerable to repeated inflammatory insult.

The cellular paradigm of chlamydia pathogenesis [13] states that the host response to chlamydiae is initiated and sustained
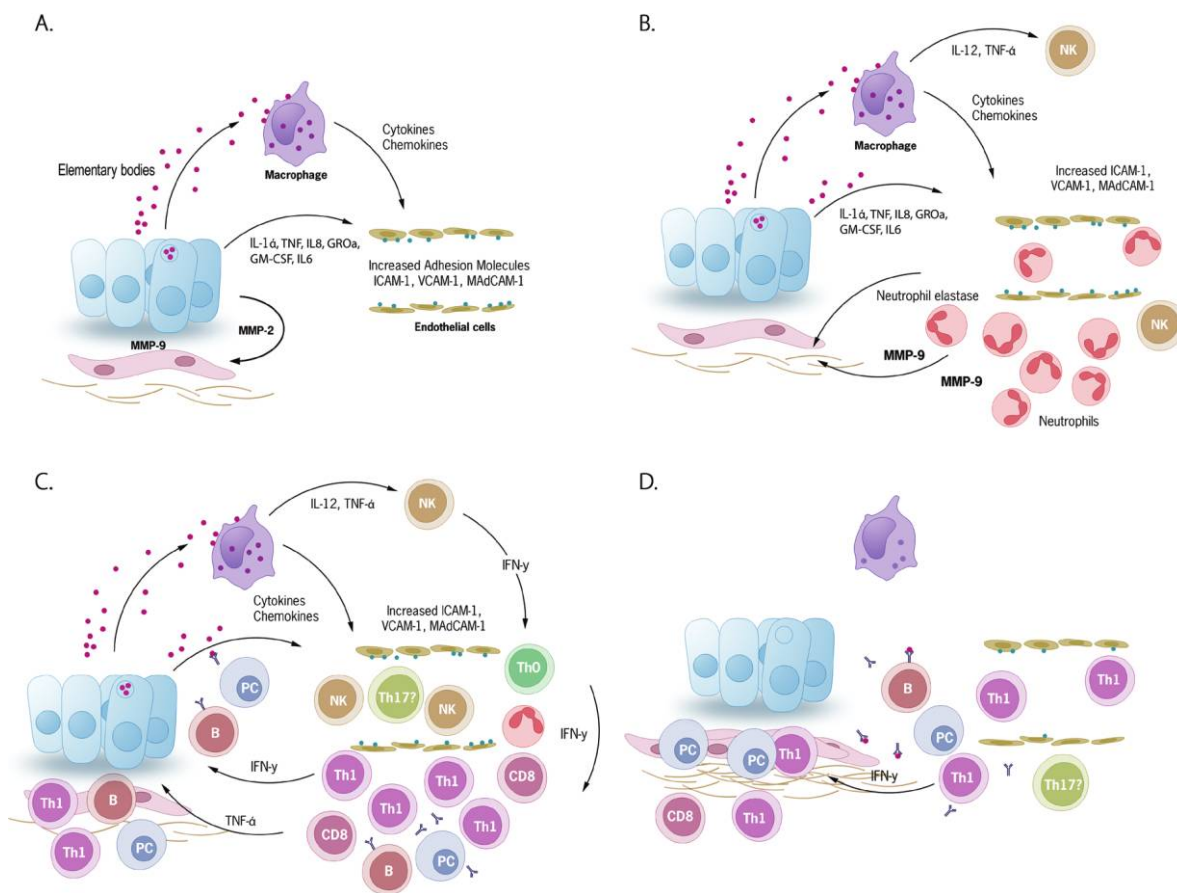

Figure 1. Infection of nonimmune host epithelial cells and resident tissue innate immune cells with chlamydiae results in production of proinflammatory cytokines and chemokines that lead to recruitment and activation of first innate and, later, adaptive immune cells to effect resolution of infection; subsets of these responses induce collateral genital tract tissue damage. *A,* Infection of reproductive tract epithelium results in production of interleuken (IL)–1, tumor necrosis factor (TNF), IL-8, growth-related oncogene (GRO)–α, granulocyte-macrophage colony stimulating factor (GM-CSF), and IL-6, which induce increased expression of endothelial adhesion molecules that aid in the attraction of immune cells. Resident tissue macrophages also contribute to early release of cytokines and chemokines. Infected epithelial cells release matrix metalloproteases (MMPs) that contribute to tissue proteolysis and remodeling. *B,* Neutrophils, natural killer (NK) cells, and monocytes are rapidly recruited into the infected tissue site. Neutrophil release of MMPs and elastase contribute to tissue damage. *C,* NK cell production of interferon (IFN)–γ drives CD4 T cells toward the Th1 (IFN-γ–producing) phenotype, and a mixture of CD4, CD8, B cells, and plasma cells (PCs) infiltrate the infected tissue. Antibodies released from PCs inactivate extracellular elementary bodies (EBs), and T cell production of IFN-γ inhibits intracellular chlamydial replication. Th17 cell involvement has not yet been determined. *D,* After infection has resolved, inflammation abates, but chronic scarring may be the end result.

by epithelial cells that are the primary targets of chlamydial infection. Infected host epithelial cells act as first responders, initiating and propagating immune responses. They secrete chemokines that recruit inflammatory leukocytes to the site of infection and cytokines that induce and augment the cellular inflammatory response [14], and these mediators induce direct damage to the tissues. At the time of reinfection, host cell release of chemokines leads to recruitment of chlamydia-specific immune cells that rapidly amplify the response. The release of proteases, clotting factors, and tissue growth factors from infected host cells and infiltrating inflammatory cells leads to tissue damage and eventual scarring—the hallmark of chlamydia-induced oviduct disease. The cellular paradigm makes no distinction between damage induced by professional innate immune cells (neutrophils and monocytes) and adaptive lymphocyte populations but assumes that both cell populations contribute to pathogenesis. Chronic chlamydial infections are common [15] and would lead to ongoing release of mediators that promote continued influx of inflammatory cells, damage to host epithelium, scarring, and ultimately, fibrosis and scarring. Because reinfection with chlamydiae is a frequent occurrence [16], repeated inflammatory responses may lead to repeated insult to the tissues and may promote tissue scarring.

Studies using the murine model of *C. trachomatis* genital tract infection have established that resolution of genital chlamydial infection is dependent on an influx of interferon (IFN)-γ–producing $CD4^+$ Th1 cells [8, 17–20]. The immunological paradigm for pathogenesis is based on the premise that T cell responses that are essential to host defense may also cause collateral tissue damage [21]. It was speculated that host T cell responses induced on primary infection to a species-specific antigen were increased with subsequent infections, promoting tissue damage and scarring [22]. Chlamydia heat shock protein 60 (Chsp60) has been investigated as a potential antigen responsible for induction of delayed type hypersensitivity–induced disease. This molecule attracted attention as a candidate pathogenic antigen after studies in immune guinea pigs and monkeys suggested direct eye inoculation with this sensitizing antigen promoted heightened inflammation of the conjunctiva [23, 24]. However, residual Triton-X detergent contaminating the extracts proved to be the inducer of disease. Later studies conducted in the guinea pig model of trachoma revealed a protective role for vaccination with Chsp60 [25], and although human studies have revealed detection of elevated titers of antibody to Chsp60 in those with more severe disease [26, 27], this may simply reflect increased exposure to chlamydia through chronic or repeated infection. A recent large prospective study of women with PID did not reveal a correlation of increased antibody titers to Chsp60 with worse outcome [28]. Furthermore, IFN-γ production by peripheral blood T cells stimulated with Chsp60 predicts protection from incident infection in women at high risk of repeated infection [29].

Despite the lack of evidence for a specific chlamydial pathogenic antigen that primes anamnestic T cell responses, in the presence of chronic or repeated infection, an ongoing or augmented memory T cell response might heighten disease development. Monkey [30] and guinea pig [31] models of repeated infection indicate that CD4 and CD8 T cells infiltrate more rapidly and in larger numbers than do neutrophils during repeat oviduct infections, and this recurrent inflammatory reaction ultimately culminates in fibrosis and scarring. Of importance, because guinea pigs develop sufficient immunity after primary infection to significantly limit bacterial burden during a secondary vaginal infection, this indicates that very small amounts of chlamydiae may be sufficient to induce an enhanced T cell response in the oviduct that culminates in disease. Although CD4 and CD8 T cells have been observed in both of these models of repeated infection, with CD8 T cells being predominant in the monkey model, no data exist on the specific role of CD8 T cells in pathogenesis in humans.

Because the ultimate goal of chlamydia control programs is to prevent reproductive tract complications, a more complete understanding of how *C. trachomatis* infection leads to sequelae is needed. The cellular paradigm of pathogenesis does not invoke professional innate or adaptive immune cells as being more or less responsible for disease development. Instead, the central player of pathogenesis is assumed to be the host epithelial cell that drives the inflammatory response through its recognition of chlamydia infection. Epithelial cells possess surface and intracellular innate immune receptors that enable them to recognize conserved chlamydial ligands and initiate inflammation. Thus, the infected epithelial cell serves as a key innate responder cell. Therefore, a determination of genetic polymorphisms that result in heightened innate inflammatory responses to chlamydia may serve to identify persons at high risk of disease development and in need of increased levels of screening and treatment. Furthermore, because tissue-damaging responses begin as soon as the bacterium infects the oviduct epithelium and the infected epithelial cells will continue to drive inflammation as long as the pathogen remains, control programs should seek to provide treatment before infection of the oviduct occurs or to shorten the duration of oviduct infection as much as possible.

The long-term morbidity associated with chlamydial infection primarily results from tissue damage at the level of the oviduct. The cellular paradigm imposes a prerequisite for infection of the oviduct to occur for disease to develop. Thus, an adaptive immune response (induced by infection at the cervix or by vaccination) that prevents ascension of bacteria to the oviduct after initial or repeated infection would effectively prevent disease. This has important implications for the design

of screening and vaccine control programs for women, because it indicates that infection of the cervix does not correlate with risk of disease as long as a strong adaptive immune response is documented in the same patient. Therefore, a vaccine that induces responses potent enough to prevent oviduct infection while containing infection to the cervix might also be effective in preventing disease sequelae. A determination of responses that predict protection from reinfection in women at high risk because of exposure is a realistic goal. If these responses could be demonstrated to be the same as those that protect an individual from infection of the upper genital tract, these responses could be used as markers of women at low risk of infection and disease. Women without such markers would be considered to be at high risk and in need of an increased frequency of screening. Because ample data indicate that chlamydia-specific CD4 Th1-IFN-γ–producing cells are key mediators of host defense, a goal for vaccine development should be to determine chlamydia antigens and adjuvants that induce a strong CD4 Th1 memory response. Of importance, the induction of sterilizing immunity would not be required to protect women from chlamydia disease.

On the other hand, if as suggested by the immunological paradigm, a small antigenic insult at the site of the oviduct after rechallenge induces tissue-damaging memory T cell responses, it would be essential for a vaccine to prevent even minimal infection of the oviduct. Thus, sterilizing immunity will be required for a vaccine to be protective. In this instance, chlamydia control programs would have to pay vigorous attention to preventing repeat infection in women with evidence of a strong CD4 Th1 memory response.

A review of published studies revealed possible avenues to the identification of specific cytokine and cellular responses that predict sequelae. Determination of biomarkers of disease will allow control efforts to be intensified for individuals identified at highest risk. We reviewed in vitro, animal, and human data related to the pathogenesis of chlamydial disease to better understand how reproductive sequelae result from *C. trachomatis* infection. We approached our review of the literature with several specific questions in mind.

WHICH INFLAMMATORY AND/OR IMMUNE RESPONSES OCCUR DURING AN INITIAL *C. TRACHOMATIS* INFECTION AND HOW DO THEY LEAD TO PATHOLOGY?

The concept of the epithelial cell as an important and early component of the host response to chlamydial infection was first revealed by Rasmussen et al [14], who showed that in vitro infection of cervical and colonic epithelial cells with *C. trachomatis* induced the secretion of an array of proinflammatory cytokines that have chemoattractant and proinflammatory functions. Moreover, in contrast to invasion by other bacteria

that induce a rapid but transient proinflammatory cytokine response at entry [32], chlamydial invasion alone was not sufficient to elicit a response. Instead, intracellular chlamydial growth was required, and the epithelial cytokine response was sustained throughout the chlamydial developmental cycle. Endocervical epithelial cells released interleuken (IL)–1α after infection, and the induced proinflammatory cytokine cascade could be inhibited by specific anti–IL-1α antibodies [14]. Thus, IL-1α, released on epithelial cell lysis, may act to amplify the inflammatory response by stimulating additional cytokine production. These findings formed the basis for Richard Stephens' [13] "cellular paradigm of chlamydial pathogenesis." Stephens theorized that "the inflammatory processes of chlamydial pathogenesis are elicited by infected host cells and are necessary and sufficient to account for chronic inflammation and the promotion of cellular proliferation, tissue remodeling and scarring—the ultimate cause of disease sequelae" [13, p 44].

Since the article by Rasmussen et al [14], others have confirmed the elicitation of inflammatory mediators from nonimmune host cells infected in vitro with *C. trachomatis*. Infection of Fallopian tube organ cultures results in epithelial cell release of IL-1 and cellular damage, independent of inflammatory cell influx. The addition of IL-1 receptor antagonist to the cultures completely eliminates tissue destruction induced by infection, indicating a direct role for this cytokine in pathogenesis [33]. In vitro infection of Fallopian tube epithelium also results in the production of tumor necrosis factor (TNF) [34] and increased expression of adhesion molecules on oviduct endothelial cells [35]. This milieu could easily lead to activation and recruitment of first innate and, later, adaptive immune cells to effect resolution of infection. However, subsets of these responses may also induce collateral damage to genital tract tissue.

Proinflammatory chemokines and cytokines have been documented in murine and guinea pig models of genital tract chlamydial infection [4, 36, 37]. The up-regulation of integrins in the murine genital tract is coincident with the onset of infection [2]. Detection of the neutrophil chemokine macrophage inflammatory protein-2 (MIP-2), a chemokine analogous to IL-8 in humans, in genital tract secretions of infected mice coincides with a rapid influx of neutrophils into the lower genital tract. Thus, data to date suggest that the inflammatory response to chlamydiae begins and is sustained by actively infected nonimmune host epithelial cells. Defining the specific responses that promote tissue damage and differentiating them from those that lead to benign resolution of infection is an important ongoing research goal.

Murine genital tract studies indicate that the intensity of neutrophil influx into the oviduct (pyosalpinx) correlates directly with eventual development of hydrosalpinx [38]. Prolonged infiltration of neutrophils into the oviduct correlates

with an increased incidence of severe hydrosalpinx in an immunologically normal mouse strain that exhibits increased disease susceptibility [39, 40]. Data by Morrison et al [8] revealed that genital chlamydial infection of mice with disrupted β2-microglobulin, I-A, or CD4 genes resulted in tubal ectasia and hydrosalpinx similar to infection of immunologically intact mice. A common feature in all mouse strains tested was the accompaniment of ascending infection of the genital tract with a marked neutrophilic inflammatory response. In class II$^{-/-}$ mice, a persistent prominent acute-subacute inflammatory response failed to resolve infection but led to development of hydrosalpinx. Although these data do not exclude a role for adaptive immune responses in the development of pathology, they indicate that chronic genital tract tissue damage can occur independently of T cell responses and implicates a role for acute inflammatory cells in its development. Activation of phagocyte oxidase in myeloid cells causes release of superoxide molecules that are damaging to cells and tissues. Mice with a deletion of a key component of phagocyte oxidase (p47phox$^{-/-}$) sustain lower rates of hydrosalpinx [41] after chlamydial infection. Other potentially important factors are matrix metalloproteinases (MMPs), which are expressed by neutrophils and monocytes and are involved in the proteolysis and resynthesis of the extracellular matrix. Ramsey et al [39] implicated neutrophil production of MMP-9 in the development of scarring and fibrosis of the murine oviduct after chlamydial infection.

Studies in humans also indicate a role for MMPs and neutrophils in production of tissue damage. Fallopian tube epithelial cells infected in vitro with *C. trachomatis* produce MMP-2, and infected oviduct stromal cells produce MMP-9 [42]. The importance of neutrophil activation in the inflammatory process associated with *C. trachomatis* infection of the female reproductive tract was also revealed in a study of women at risk of PID in which vaginal levels of neutrophil α-defensins (HNP1–3), markers of neutrophil activation, were strongly associated with the presence of endometritis [43]. Absolute numbers of vaginal neutrophils were associated with endometritis only in the presence of elevated defensin levels. Although oculogenital serovars are killed by neutrophils, in human endometrial epithelial cell cultures monitored for >1 month after exposure to azithromycin (a chlamydiacidal antibiotic)–loaded neutrophils, residual chlamydial envelopes and major outer membrane protein and lipopolysaccharide antigens were detected and continued to stimulate neutrophil chemotaxis [44]. Thus, antigens may persist for some time after the organisms are killed, inducing continued neutrophil inflammation and ongoing release of tissue-damaging molecules in the host.

Taken together, these data imply that ascension of chlamydiae from the lower to the upper genital tract and, ultimately, infection of oviduct epithelium are prerequisites for the development of long-term sequelae. Support of this hypothesis comes from the lack of detection of oviduct pathology in mice infected vaginally with human serovars [4], where infection of the cervix and endometrium occur but oviduct infection does not [4, 45]. Human serovars elicit oviduct pathology in the mouse when they are inoculated directly under the ovarian bursa [46]. On the other hand, infection of the oviduct does not guarantee disease development. Rank and Sanders [9] documented that oviduct infection occurred in 78% of vaginally inoculated guinea pigs, but chronic oviduct pathology was seen in only 12%. Thus, additional host factors must influence the development of disease.

Researchers have begun to determine the cellular receptors involved in *C. trachomatis*–induced stimulation of cytokine release. Toll-like receptors (TLRs) act as pathogen-recognition receptors that enable cells to recognize conserved bacterial, viral, and fungal structural elements. In vitro, *C. trachomatis* infection of HEK cells transfected with the adaptor molecule MyD88 and the pathogen molecular pattern receptors TLR2 and TLR4/MD-2 revealed that TLR2 was required for IL-8 secretion and that the role of TLR4/MD-2 was minimal. This was reproduced with chlamydial infection of immortalized human ectocervical epithelial cells [47]. Activation was dependent on live, replicating bacteria, because infection with ultra violet–irradiated bacteria and treatment of infected cells with chloramphenicol (no inclusions), but not ampicillin (jammed in reticulate body form), abrogated the induction of IL-8 secretion. The response was largely dependent on the MyD88 adaptor molecule. Confocal microscopy experiments revealed that both TLR2 and MyD88 colocalize with the intracellular chlamydial inclusion, suggesting that TLR2 is actively engaged in signaling from this intracellular location.

Examination of the outcomes of genital tract infection in wild-type mice and mice genetically deficient in TLR2 and TLR4 confirmed a dominant role for TLR2, compared with TLR4, in the recognition and response to *Chlamydia muridarum* in the genital tract [48]. TLR4-/- mice responded to infection similarly to wild-type controls and developed similar pathology. Eradication of lower genital tract infection was equivalent in TLR2-/- mice and wild-type mice, even in the early days after infection. However, significantly lower levels of TNF-α and MIP-2 were detected in genital tract secretions of TLR2-/- mice during the first week of infection, and there was a significant reduction in oviduct and mesosalpinx pathology at later times. Thus, the cytokine response that remained in the absence of TLR2 signaling was sufficient to recruit effector cells to the genital tract, but these data indicate a protective role for TLR2 deficiency in genital tract infection sequelae due to *C. trachomatis*. Wild-type mice that are infected with plasmid-deficient *C. muridarum* that fail to signal via TLR2 develop a predominant Th1 response, and they are as resistant to secondary challenge infection as immunologically normal mice

primarily infected with wild-type *C. muridarum* [49]. Thus, although pathology is abrogated, chlamydia-induced signaling that is independent of TLR2 leads to an effective acquired immune response, speaking to the redundancy of the innate immune system. The oviduct bacterial burden induced by infection with the plasmid-deficient strain of *C. muridarum,* CM3.1, is not different from that induced by infection with wild-type *C. muridarum* [49]. It is possible that the downstream immune response elicited by infection with this plasmid-deficient chlamydial strain is simply globally muted in intensity or that the quality of the response is altered and specific mediators of tissue damage are no longer induced. Regardless of the mechanism, these data confirm the ability of the host epithelial cell–initiated response to induce downstream immune effectors that eradicate oviduct infection without causing chronic pathology and prevent pathology after secondary infection.

Examination of human tissue samples for the various TLRs has revealed mRNA for TLRs1–9 in uterine epithelium [50]. TLR2 is highly expressed in Fallopian tubes and the cervix [51], whereas TLR4 is weakly expressed in Fallopian tubes [51] and mRNA for TLR4 and its coadaptor MD2 is not detected in human cervical epithelial cells [52]. Thus, by virtue of its relative expression, TLR2 may be a primary pathogen-recognition receptor available in the lower genital tract and oviducts to drive the pathology-inducing inflammatory response to chlamydial infection.

There are only a few published studies of the histopathology of *C. trachomatis*–infected human reproductive tract tissue. Examination of human endocervical biopsy samples from girls and women with mucopurulent cervicitis due to *C. trachomatis* revealed a focal loss of surface columnar epithelial cells, intrepithelial neutrophils, and glandular intraluminal neutrophils [53]. Dense subepithelial plasma cell infiltrates and well-formed periglandular germinal centers were also noted. The underlying tissue architecture was maintained. Lymphocytes and histiocytes were present in the stroma but rarely outnumbered plasma cells. Aggregation of neutrophils around cells positive for *C. trachomatis* by immunofluorescence implies a prominent role for neutrophils in the response. In a study involving women with clinically suspected PID, Kiviat et al [1] attempted to determine the relationship of histologic endometrial abnormalities with documented oviduct infection and laparoscopically proven acute salpingitis. The best predictor of salpingitis, without discrimination to organism, was the simultaneous presence of >5 neutrophils per ×400 field in endometrial surface epithelium, together with ≥1 plasma cell per ×120 field in the endometrial stroma. Features discriminatory for *C. trachomatis* infection included higher numbers of plasma cells and more periglandular lymphoid follicles containing transformed lymphocytes. Infiltration of glandular epithelium by neutrophils was observed in patients with documented endometrial and oviduct infection with *C. trachomatis.* In addition, germinal center formation in subepithelial tissue was commonly noted. The prominence of both acute and chronic inflammatory cells in infected human female genital tract tissues does not assist in the determination of specific cell types responsible for disease sequelae. The participants in these studies were recruited from sexually transmitted diseases clinics; the mean age in the endocervical biopsy study was 22 years and in the endometrial biopsy study was 23 years. Thus, it is likely that at least a proportion of patients in both groups had a history of *C. trachomatis* infection.

It is difficult to draw conclusions about the cause and effect nature of cellular infiltrates noted in a single biopsy specimen from an individual infected for an unknown duration. No studies have documented the histology of infection before and after antibiotic treatment; ethical concerns prevent the performance of sequential analyses of tissue specimens from infected persons. In addition, without documentation of negative serologic examination results antecedent to the diagnosis of infection, the primary versus repeated nature of the infection is extremely difficult to delineate.

Germinal center formation generally depends on cooperative interactions of helper T cells and B cells. Helper T cells activated by antigen-presenting dendritic cells express the surface molecule CD40 ligand and secrete cytokines. CD40 ligand on activated helper T cells then binds to CD40 on B cells and initiates B cell proliferation and differentiation. After a few days of antigen exposure, a single lymphocyte within a germinal center may give rise to 5000 progeny. Thus, the presence of follicles and germinal centers indicates local stimulation of adaptive immune responses occurs in infected female genital tract tissues. Whether follicles persist after infection has resolved is unknown. One might speculate that this local proliferation of adaptive immune cells would enhance resolution of infection and prevent pathology. However, as discussed below, data from models of repeated infection in guinea pigs and monkeys indicate the association of such lymphocytic responses with pathology.

DURING REPEAT INFECTIONS, WHICH IMMUNE RESPONSES DETERMINE PATHOLOGY AND HOW?

Determination of which immune responses associated with repeated infection determine pathology is difficult, because histologic studies involving humans have revealed both acute and chronic inflammatory cell infiltrates [1]. Monkey and guinea pig models of repeated infection have revealed that an early acute inflammatory response occurs with a similar magnitude in both initial and repeat infections, whereas T cells infiltrate more rapidly and in larger numbers in repeat infections than in primary infection [54, 55]. The recurrent inflammatory re-

action ultimately culminates in fibrosis and scarring [56]. Primary infection in the mouse model induces such severe oviduct pathology that this model is somewhat impractical for addressing the role of specific cell types in induction of pathology with repeat infection.

Reinfection in the macaque salpingeal pocket model resulted in a rapid lymphocytic infiltration, resulting in notable epithelial cell destruction and the formation of lymphoid follicles [55]. Tertiary infection of pockets reignites lymphocytic activity with focal areas of epithelium destruction and the formation of extensive fibrosis and lymphoid follicles in the deep stroma [30]. Additional data from this model indicate that the robust inflammatory response to repeat infection may be mediated by cytotoxic CD8 T cells primed against Chsp60 [57]. Thus, this direct inoculation nonhuman primate model displays immune-mediated destruction of genital tract tissue that is enhanced with each repeat infection. Human epidemiologic studies have indicated increased risk of disease with repeated infection [58–60]. However, this may simply be a cumulative process rather than the result of enhanced destruction with each repeat infection, as seen in this primate model. In addition, it should be noted that these models differ from human infections in that the oviducts are infected directly rather than by ascension from the lower genital tract. During natural infection, the time required for ascension of the bacterium to the oviducts may allow for homing of protective memory T cells, leading to a decrease in the infectious inoculum and consequent damaging inflammation at this vulnerable tissue site. In addition, animals are not treated with antibiotics between infections; thus, these data cannot be extrapolated to humans who sustain repeat infection after treatment of an initial infection.

Primary vaginal infection of guinea pigs with *Chlamydia caviae* (formally known as the guinea pig inclusion conjunctivitis serovar of *Chlamydia psittaci*) leads to an ascending infection that is cleared by ∼20 days after infection [9]. Although infection of at least 1 oviduct can be documented in most animals after a primary infection, less than half develop notable tubal pathology. Both cellular and humoral immune mechanisms generated in a primary infection protected animals from reinfection for up to 30 days; however, this immunity waned such that animals were susceptible beginning on day 77 [61–63]. Although secondary infections were noted to be markedly abbreviated with a significant reduction in bacterial burden, an increased number of animals developed oviduct dilatation with repeat infection [31]. One potential caveat is that oviduct pathology was evaluated 105–112 days after primary infection in challenged animals and 75–85 days after primary infection in nonchallenged animals. It is possible that increased scarring occurred over time, leading to the detection of an increased frequency of oviduct dilation in the challenged animals. However, definite enhancement of cell-mediated inflammation after

rechallenge was observed in a subsequent study using the guinea pig model in which flow cytometric analysis revealed that significantly higher levels of T and B cells, but not Mac-1–positive cells, were recruited to the oviduct in rechallenged animals, compared with animals infected only once [64]. Of interest, little to no *C. caviae* reached the oviduct during repeat infection, suggesting that this organ is exceptionally susceptible to a small antigenic insult after priming through an initial infection and providing strong evidence for a cell-mediated mechanism of pathology in repeat infection. Further development of this model and the use of guinea pig–specific reagents may provide a useful surrogate to investigate the possibility that repeat infection leads to an accelerated inflammatory response resulting in enhanced disease.

WHAT IS THE ROLE OF HOST FACTORS IN PATHOGENESIS? ARE THERE RELIABLE MARKERS OF PATHOGENESIS OR PATHOLOGY?

Studies have suggested an association between specific host immune responses and susceptibility to chlamydial infection and/or disease (Table 1). In a prospective cohort study involving women at high risk of *C. trachomatis* infection, Cohen et al [29] found that, at baseline and after adjustment for age and other potential confounding variables, production of IFN-γ by peripheral-blood mononuclear cells (PBMCs) stimulated with Chsp60 strongly correlated with protection against incident *C. trachomatis* infection, although there was no information regarding development of sequelae. In a small study involving Australian women attending a sexually transmitted diseases clinic, Debattista et al [65] found that low PBMC IFN-γ and high IL-10 responses to Chsp60 were markers for increased risk of chlamydial infection and PID. In human immunodeficiency virus–seropositive women, a CD4 lymphocyte count of <400 cells/mm^3 was determined to be an independent risk factor for *C. trachomatis* PID (odds ratio, 21.7; 95% confidence interval, 1.2–383; $P = .036$) [58]. These studies support a role for chlamydia-responsive CD4 Th1–IFN-γ–producing cells in protection from disease. In women with chlamydia-related tubal infertility, T cell responses to Chsp60 were associated with a specific IL-10 promoter polymorphism (IL-10–1082AA) and with specific HLA class II DQ alleles (HLA-DQA1*0102 and HLA-DQB1*0602) [66]. Thus, genetic factors that regulate the induction and activation of Th1 cells may be important in directing a protective host response.

A recent study by Agrawal et al [67] examined cervical lymphocyte cytokine responses of 255 *C. trachomatis* antibody–positive women with or without fertility disorders (infertility and multiple spontaneous abortions) and of healthy control women negative for *C. trachomatis* serum IgM or IgG. Flow cytometric analysis revealed a significant increase in the mean

Table 1. Host Factors and Cellular Immune Responses Associated with Susceptibility or Protection from Infection and/or Disease

Host factor or response	Association	Reference(s)
IFN-γ production by PBMCs stimulated with Chsp60	Protection from incident *C. trachomatis* infection	[29]
Low PBMC IFN-γ and high IL-10 responses to Chsp60	Increased risk of *C. trachomatis* infection and PID	[65]
Low CD4 cell count in HIV-infected women	Increased risk of PID	[58]
Neutrophils in cervical secretions	Positive correlation with histologic endometritis in girls with clinical PID	[79]
Cervical neutrophil defensin levels	Positive correlation with histologic endometritis in girls at risk of PID	[43]
IL-10 promoter polymorphism (IL-10–1082AA); HLA class II DQ alleles (HLA-DQA1*0102 and HLA-DQB1*0602)	Increased risk of TFI	[66]
Cervical cell production of IL-1β, IL-6, IL-8, and IL-10 in response to stimulation with *Chlamydia trachomatis* EBs	Positive correlation with infertility in *C. trachomatis*–seropositive patients	[67]
Cervical cell production of IFN-γ and IL-12 in response to stimulation with *C. trachomatis* EBs	Positive correlation with fertility in *C. trachomatis*–seropositive patients	[67]
T cell chemokine receptor deletion mutation CCR5-Δ32	Negative correlation with tubal damage among *C. trachomatis* seropositive patients	[68]
Functional genetic polymorphisms for TLR4, CD14, IL-1b, and the IL-1 receptor	No association with TFI	[74–76]
Functional genetic polymorphism for MBL	Negative correlation with TFI	[77]

NOTE. Chsp60, *Chlamydia* heat-shock protein 60; EBs, elementary bodies; IFN, interferon; MBL, mannose binding lectin; PID, pelvic inflammatory disease; PBMCs, peripheral blood mononuclear cells; TFI, tubal factor infertility.

number of CD4+ T lymphocytes in the cervical mucosa of fertile women, compared with those with fertility disorders and with negative control women. The CD8+ T cell population was found to be only slightly increased in the cervical mucosa during chlamydial infection in both groups. Cervical cells from the women with fertility disorders secreted significantly higher levels of IL-1β, IL-6, IL-8, and IL-10 in response to in vitro stimulation with *C. trachomatis* elementary bodies; whereas, cervical cells from antibody-positive fertile women secreted significantly higher levels of IFN-γ and IL-12. Reverse-transcription polymerase chain reaction analysis confirmed these results for IFN-γ and IL-12. Gene expression levels for IL-10 and IL-4 were significantly higher in cells obtained from women with fertility disorders. These data suggest that human chlamydia-responsive mucosal lymphocytes skewed toward Th1 predominance protect from chronic sequelae.

In contrast to the above data, a study by Barr et al [68] provides genetic support for the role of tissue-infiltrating memory T cells in pathogenesis. Although the study was limited by a sample size of only 41 *C. trachomatis*–seropositive patients, the chemokine receptor deletion mutation CCR5-Δ32 correlated significantly with protection from tubal damage. Chemokine receptor 5 is crucial for T cell activation and function. These same investigators compared the outcome of chlamydial genital infection in wild-type mice with that in mice genetically deficient for CCR5 and determined that, although the mice deficient in CCR5 sustained an infection course of greater in-

tensity and duration, they were protected from infertility, compared with immunologically intact mice [68]. These data reveal the potential double-edged effect of T cell effectors activated in response to chlamydial infection.

Investigations of the CD4 T cell lineage Th17 may help to explain these conflicting data. The Th17 response has been implicated in the development of infection-induced immunopathology in other models of infection [69, 70]. Th17 cells produce IL-17, which recruits neutrophils to inflammatory sites and induces neutrophil-promoting chemokines. Cytokines that influence Th1 and Th2 lineage commitment, specifically IFN-γ and IL-4, inhibit the development of Th17 cells [71, 72]. Furthermore, IL-1, which is released from infected epithelial cells, has been shown to promote Th17 differentiation [73]. In light of the role played by Th17 cells in the activation of neutrophils and the recognition that the Th17 response is dampened by IFN-γ, it is possible that chlamydia-specific Th1 cell production of IFN-γ acts to inhibit disease-promoting Th17 responses. Low production of IFN-γ would allow for increased Th17 responses and increased disease.

Limited studies involving white Dutch women that examined human genetic functional polymorphisms related to the innate immune molecules, TLR4 [74], the lipopolysaccharide antigen–sensing TLR4 coreceptor CD14 [75], IL-1β, and the IL-1 receptor [76] have thus far revealed no association of functional polymorphisms in genes for these molecules and tubal infertility. In contrast, wild-type homozygosity for mannose-binding

lectin allele A has been associated with protection against tubal occlusion in white Hungarian women [77]. Binding of mannose-binding lectin to sugar groups of the *C. trachomatis* major outer membrane protein blocks attachment of organisms to host cells [78]. Thus, low levels of mannose-binding lectin may lead to an increased infectious burden and/or increased ascension to the oviducts, resulting in increased risk for tubal factor infertility. Examination for functional polymorphisms in genes for TLR2 (shown to be essential for induction of oviduct pathology in the mouse model) and the TLR2 coreceptors TLR1 and TLR6 with use of banked specimens from women with and without infertility previously enrolled in the PID evaluation and clinical health study (PEACH) [28] are ongoing (T. Darville and C. L. Haggerty, personal communication).

Researchers have attempted to determine factors identifiable using cervical samples that predict upper genital tract pathology. As reported above, vaginal neutrophil defensin levels have been shown to correlate with presence of endometritis in women with subclinical PID [43]. An earlier study revealed a strong correlation between detection of neutrophils in cervical secretions and presence of endometritis as determined by examination of biopsy samples from girls with clinically symptomatic PID [79]. However, it remains to be determined whether leucorrhea and/or elevated cervical mucus defensin levels predict salpingitis and/or increased risk of chronic tubal damage.

One important question is that of the relationship of antichlamydial antibodies and disease risk. Risk factors for *C. trachomatis* PID that were established in prospective studies involving female sex workers include positive serum antibody to Chsp60 [58, 80] and repeated *C. trachomatis* infection [58]. Among women with mild to moderate PID, antibody titers to *C. trachomatis* elementary bodies measured in the highest tertile at follow-up, but not to Chsp60, were independently associated with elevated rates of recurrent PID and reduced rates of pregnancy [28]. Although these studies linked positive antibody responses to chlamydial proteins to sequelae, these responses may simply be markers for increased exposure to chlamydiae. Increased exposure could be in the form of repeated or chronic infection. Monkeys inoculated in their oviducts with *C. trachomatis* and subsequently treated with antibiotics were examined for antibodies to Chsp60. A persistent Chsp60 antibody response was correlated with having culture- or ligase chain reaction–positive oviduct samples after treatment, which suggests that antibody positivity is a useful marker of chronic infection [81]. These data indicate that prolonged or repeated exposure to chlamydiae leads to increased risk for disease and increased detection of anti-chlamydial antibodies, rather than implicating antibody formation directly in pathogenesis.

Another possibility is that female individuals who are prone to develop high titers of antibody (Th2) are less likely to develop a protective cell-mediated (Th1) immune response. Although high antibody responses to Chsp60 have been correlated with increased susceptibility to chlamydial PID [58, 80], IFN-γ responses to this highly conserved protein have been correlated with protection among the same group of women [58]. Although logistically very difficult, more correlative longitudinal data on antibody and T cell responses are needed in adolescent and adult women who sustain an initial or repeated infection.

SUMMARY AND CONCLUSIONS

The most conclusive pathogenesis data to date reveal that actively infected nonimmune host cells are the driving force in the inflammatory response to chlamydia. In vitro and mouse data indicate that infected host epithelial cells not only drive influx of inflammatory cells, but also release tissue-damaging molecules directly. Mouse and in vitro data implicate neutrophils and proteinases released from activated neutrophils in tissue pathogenesis and indicate an important role for chlamydia-induced activation of TLR2 in initiation of these responses. However, continued release of chemokines from infected host cells drives recruitment of not only innate neutrophils and monocytes, but also adaptive T cells and B cells. The intracellular niche of chlamydia makes it logical that T cell responses would be important for successful eradication of infection, and multiple studies involving mice and humans suggest that the CD4 Th1 response is the principle mechanism of host defense. Thus, a key question that needs to be answered is whether anamnestic CD4 Th1 responses induced after reinfection, with ongoing chronic infection, or after vaccination cause disease.

Limited evidence from animal models indicates that the adaptive T cell response may promote pathology, especially with ongoing chronic or repeat infection. However, recent vaccine studies in mice indicate that induction of a Th1 immune response to vaccine antigens is essential for the vaccine to protect challenged animals from disease [82, 83]. In addition, human immunoepidemiologic studies have shown that women at high risk of exposure that develop chlamydia-specific CD4^{+} Th1–IFN-γ–producing cells are protected from incident infection [29]. Because it is unlikely that a vaccine that induces primarily antibody responses will be effective against a pathogen that replicates in a protective vacuole intracellularly, conclusive evidence that induction of Th1 immunity is solely protective is crucial for vaccine development.

Although CD8 T cells have been detected in guinea pig and monkey models of repeat infection in association with oviduct disease, the role of these cells in human disease needs to be defined. In addition, chlamydia-specific Th17 cells should be examined as potential mediators of pathogenesis. These cells activate potentially tissue-damaging neutrophils, and their ac-

tivation has been associated with increased inflammation and disease in other infectious disease models.

The application of multicolor flow cytometric methods to determine specific cell types, cell function, and the kinetics of these responses in genital tract tissues with use of established animal models should assist in answering these questions. These same techniques can be applied to human specimens, such as peripheral blood, endocervical brush, and endometrial biopsy samples, in conjunction with collection of clinical and epidemiological data. Furthermore, knowledge gained from the use of genetically engineered knock-out mouse models related to pathogen-host cell interactions that stimulate disease-inducing immune responses should be investigated in humans. Elucidation of specific cytokine and cellular responses as predictors of sequelae would allow control efforts to be intensified for individuals identified to be at highest risk of disease. Approaches include examinations conducted with human cells and tissue samples ex vivo and large-scale immunoepidemiologic, immunopathologic, and genetic studies correlated with clinical data on infection and disease status.

Acknowledgments

References

1. Kiviat NB, Wolner-Hanssen P, Eschenbach DA, et al. Endometrial histopathology in patients with culture-proved upper genital tract infection and laparoscopically diagnosed acute salpingitis. Am J Surg Pathol 1990; 14(2):167–175.
2. Kelly KA, Rank RG. Identification of homing receptors that mediate the recruitment of CD4 T cells to the genital tract following intravaginal infection with *Chlamydia trachomatis.* Infect Immun 1997; 65(12): 5198–5208.
3. Morrison SG, Morrison RP. In situ analysis of the evolution of the primary immune response in murine Chlamydia trachomatis genital tract infection. Infect Immun 2000; 68(5):2870–2879.
4. Darville T, Andrews CW Jr, Laffoon KK, Shymasani W, Kishen LR, Rank RG. Mouse strain-dependent variation in the course and outcome of chlamydial genital tract infection is associated with differences in host response. Infect Immun 1997; 65(8):3065–3073.
5. Soloff BL, Rank RG, Barron AL. Ultrastructural studies of chlamydial infection in guinea pig urogenital tract. J Comp Pathol 1982; 92: 547–558.
6. Rank RG, Barron AL. Effect of antithymocyte serum on the course of chlamydial genital infection in female guinea pigs. Infect Immun 1983; 41(2):876–879.
7. Ramsey KH, Rank RG. Resolution of chlamydial genital infection with antigen-specific T- lymphocyte lines. Infect Immun 1991; 59(3): 925–931.
8. Morrison RP, Feilzer K, Tumas DB. Gene knockout mice establish a primary protective role for major histocompatibility complex class II-restricted responses in *Chlamydia trachomatis* genital tract infection. Infect Immun 1995; 63(12):4661–4668.
9. Rank RG, Sanders MM. Pathogenesis of endometritis and salpingitis in a guinea pig model of chlamydial genital infection. Am J Pathol 1992; 140(4):927–936.
10. Paavonen J, Eggert-Kruse W. *Chlamydia trachomatis:* impact on human reproduction. Hum Reprod Update 1999; 5(5):433–447.
11. van Valkengoed IG, Morre SA, van den Brule AJ, Meijer CJ, Bouter LM, Boeke AJ. Overestimation of complication rates in evaluations of Chlamydia trachomatis screening programmes–implications for cost-effectiveness analyses. Int J Epidemiol 2004; 33(2):416–425.
12. World Health Organization. Global prevalence and incidence of selected curable sexually transmitted diseases: overview and estimates. Geneva: world Health Organization, 2001:1–34.
13. Stephens RS. The cellular paradigm of chlamydial pathogenesis. Trends Microbiol 2003; 11:44–51.
14. Rasmussen SJ, Eckmann L, Quayle AJ, et al. Secretion of proinflammatory cytokines by epithelial cells in response to *Chlamydia* infection suggests a central role for epithelial cells in chlamydial pathogenesis. J Clin Invest 1997; 99(1):77–87.
15. Molano M, Meijer CJ, Weiderpass E, et al. The natural course of *Chlamydia trachomatis* infection in asymptomatic Colombian women: a 5-year follow-up study. J Infect Dis 2005; 191(6):907–916.
16. Burstein GR, Gaydos CA, Diener-West M, Howell MR, Zenilman JM, Quinn TC. Incident *Chlamydia trachomatis* infections among inner-city adolescent females [see comments]. JAMA 1998; 280(6):521–526.
17. Cain TK, Rank RG. Local Th1-like responses are induced by intra-vaginal infection of mice with the mouse pneumonitis biovar of *Chlamydia trachomatis.* Infect Immun 1995; 63(5):1784–1789.
18. Perry LL, Feilzer K, Caldwell HD. Immunity to *Chlamydia trachomatis* is mediated by T helper 1 cells through IFN-gamma-dependent and -independent pathways. J Immunol 1997; 158(7):3344–3352.
19. Cotter TW, Ramsey KH, Miranpuri GS, Poulsen CE, Byrne GI. Dissemination of *Chlamydia trachomatis* chronic genital tract infection in gamma interferon gene knockout mice. Infect Immun 1997; 65(6): 2145–2152.
20. Morrison SG, Su H, Caldwell HD, Morrison RP. Immunity to murine *Chlamydia trachomatis* genital tract reinfection involves B cells and CD4(+) T cells but not CD8(+) T cells. Infect Immun 2000; 68(12): 6979–6987.
21. Brunham RC, Rey-Ladino J. Immunology of Chlamydia infection: implications for a *Chlamydia trachomatis* vaccine. Nat Rev Immunol 2005; 5(2):149–161.
22. Grayston JT, Wang SP, Yeh LJ, Kuo CC. Importance of reinfection in the pathogenesis of trachoma. Rev Infect Dis 1985; 7(6):717–725.
23. Watkins NG, Hadlow WJ, Moos AB, Caldwell HD. Ocular delayed hypersensitivity: a pathogenetic mechanism of chlamydial conjunctivitis in guinea pigs. Proc Natl Acad Sci U S A 1986; 83:7480–7484.
24. Morrison RP, Lyng K, Caldwell HD. Chlamydial disease pathogenesis: ocular hypersensitivity elicited by a genus-specific 57-kD protein. J Exp Med 1989; 169(3):663–675.
25. Rank RG, Dascher C, Bowlin AK, Bavoil PM. Systemic immunization with Hsp60 alters the development of chlamydial ocular disease. Invest Ophthalmol Vis Sci 1995; 36(7):1344–1351.
26. Peeling RW, Kimani J, Plummer F et al. Antibody to chlamydial hsp60 predicts an increased risk for chlamydial pelvic inflammatory disease. J Infect Dis 1997; 175(5):1153–1158.
27. Toye B, Laferrière C, Claman P, Jessamine P, Peeling R. Association between antibody to the chlamydial heat-shock protein and tubal infertility. J Infect Dis 1993; 168:1236–1240.
28. Ness RB, Soper DE, Richter HE, et al. Chlamydia antibodies, chlamydia heat shock protein, and adverse sequelae after pelvic inflammatory disease: the PID Evaluation and Clinical Health (PEACH) Study. Sex Transm Dis 2008; 35(2):129–135.
29. Cohen CR, Koochesfahani KM, Meier AS, et al. Immunoepidemiologic profile of *Chlamydia trachomatis* infection: importance of heat-shock protein 60 and interferon-gamma. J Infect Dis 2005; 192(4):591–599.
30. Van Voorhis WC, Barrett LK, Sweeney YT, Kuo CC, Patton DL. Repeated *Chlamydia trachomatis* infection of Macaca nemestrina fallopian

tubes produces a Th1-like cytokine response associated with fibrosis and scarring. Infect Immun **1997**; 65(6):2175–2182.

31. Rank RG, Sanders MM, Patton DL. Increased incidence of oviduct pathology in the guinea pig after repeat vaginal inoculation with the chlamydial agent of guinea pig inclusion conjunctivitis. Sex Transm Dis **1995**; 22(1):48–54.

32. Eckmann L, Kagnoff MF, Fierer J. Epithelial cells secrete the chemokine interleukin-8 in response to bacterial entry. Infect Immun **1993**; 61: 4569–4574.

33. Hvid M, Baczynska A, Deleuran B, et al. Interleukin-1 is the initiator of fallopian tube destruction during *Chlamydia trachomatis* infection. Cell Microbiol **2007**; 9:2795–2803.

34. Ault KA, Tawfik OW, Smith-King MM, Gunter J, Terranova PF. Tumor necrosis factor-alpha response to infection with Chlamydia trachomatis in human fallopian tube organ culture. Am J Obstet Gynecol **1996**; 175(5):1242–1245.

35. Kelly KA, Natarajan S, Ruther P, Wisse A, Chang MH, Ault KA. *Chlamydia trachomatis* infection induces mucosal addressin cell adhesion molecule-1 and vascular cell adhesion molecule-1, providing an immunologic link between the fallopian tube and other mucosal tissues. J Infect Dis **2001**; 184(7):885–891.

36. Belay T, Eko FO, Ananaba GA et al. Chemokine and chemokine receptor dynamics during genital chlamydial infection. Infect Immun **2002**; 70(2):844–850.

37. Darville T, Lafoon KK, Kishen LR, Rank RG. Tumor necrosis factor-alpha activity in genital tract secretions of guinea pigs infected with chlamydiae. Infect Immun **1995**; 63:4675–4681.

38. Shah AA, Schripsema JH, Imtiaz MT, et al. Histopathologic changes related to fibrotic oviduct occlusion after genital tract infection of mice with *Chlamydia muridarum*. Sex Transm Dis **2005**; 32(1):49–56.

39. Ramsey KH, Sigar IM, Schripsema JH, Shaba N, Cohoon KP. Expression of matrix metalloproteinases subsequent to urogenital *Chlamydia muridarum* infection of mice. Infect Immun **2005**; 73(10):6962–6973.

40. Darville T, Andrews CW, Jr., Sikes JD, Fraley PL, Rank RG. Early local cytokine profiles in strains of mice with different outcomes from chlamydial genital tract infection. Infect Immun **2001**; 69(6):3556–3561.

41. Ramsey KH, Sigar IM, Rana SV, Gupta J, Holland SM, Byrne GI. Role for inducible nitric oxide synthase in protection from chronic *Chlamydia trachomatis* urogenital disease in mice and its regulation by oxygen free radicals. Infect Immun **2001**; 69(12):7374–7379.

42. Ault KA, Kelly KA, Ruther PE, et al. *Chlamydia trachomatis* enhances the expression of matrix metalloproteinases in an in vitro model of the human fallopian tube infection. Am J Obstet Gynecol **2002**; 187(5): 1377–1383.

43. Wiesenfeld HC, Heine RP, Krohn MA, et al. Association between elevated neutrophil defensin levels and endometritis. J Infect Dis **2002**; 186(6):792–797.

44. Wyrick PB, Knight ST, Paul TR, Rank RG, Barbier CS. Persistent chlamydial envelope antigens in antibiotic-exposed infected cells trigger neutrophil chemotaxis. J Infect Dis **1999**; 179(4):954–966.

45. Ito JI Jr, Lyons JM, Airo-Brown LP. Variation in virulence among oculogenital serovars of *Chlamydia trachomatis* in experimental genital tract infection. Infect Immun **1990**; 58(6):2021–2023.

46. Tuffrey M, Falder P, Taylor-Robinson D. Genital-tract infection and disease in nude and immunologically competent mice after inoculation of a human strain of *Chlamydia trachomatis*. Br J Exp Pathol **1982**; 63(5):539–546.

47. O'Connell CM, Ionova IA, Quayle AJ, Visintin A, Ingalls RR. Localization of TLR2 and MyD88 to *Chlamydia trachomatis* inclusions: evidence for signaling by intracellular TLR2 during infection with an obligate intracellular pathogen. J Biol Chem **2006**; 281(3):1652–1659.

48. Darville T, O'Neill JM, Andrews CW Jr, Nagarajan UM, Stahl L, Ojcius DM. Toll-like receptor-2, but not toll-like receptor-4, is essential for development of oviduct pathology in chlamydial genital tract infection. J Immunol **2003**; 171(11):6187–6197.

49. O'Connell CM, Ingalls RR, Andrews CW Jr, Skurlock AM, Darville T. Plasmid-deficient *Chlamydia muridarum* fail to induce immune pathology and protect against oviduct disease. J Immunol **2007**; 179(6): 4027–4034.

50. Schaefer TM, Fahey JV, Wright JA, Wira CR. Innate immunity in the human female reproductive tract: antiviral response of uterine epithelial cells to the TLR3 agonist poly(I:C). J Immunol **2005**; 174(2): 992–1002.

51. Pioli PA, Amiel E, Schaefer TM, Connolly JE, Wira CR, Guyre PM. Differential expression of Toll-like receptors 2 and 4 in tissues of the human female reproductive tract. Infect Immun **2004**; 72(10): 5799–5806.

52. Fichorova RN, Cronin AO, Lien E, Anderson DJ, Ingalls RR. Response to *Neisseria gonorrhoeae* by cervicovaginal epithelial cells occurs in the absence of toll-like receptor 4-mediated signaling. J Immunol **2002**; 168(5):2424–2432.

53. Kiviat NB, Paavonen JA, Wolner-Hanssen P, et al. Histopathology of endocervical infection caused by *Chlamydia trachomatis*, herpes simplex virus, *Trichomonas vaginalis*, and *Neisseria gonorrhoeae*. Hum Pathol **1990**; 21(8):831–837.

54. Rank RG, Bowlin AK, Kelly KA. Characterization of lymphocyte response in the female genital tract during ascending chlamydial genital infection in the guinea pig model. Infect Immun **2000**; 68(9): 5293–5298.

55. Patton DL, Kuo CC. Histopathology of *Chlamydia trachomatis* salpingitis after primary and repeated reinfections in the monkey subcutaneous pocket model. J Reprod Fertil **1989**; 85(2):647–656.

56. Rank RG, Sanders MM, Patton DL. Increased incidence of oviduct pathology in the guinea pig after repeat vaginal inoculation with the chlamydial agent of guinea pig inclusion conjunctivitis. Sex Transm Dis **1995**; 22(1):48–54.

57. Lichtenwalner AB, Patton DL, Van Voorhis WC, Sweeney YT, Kuo CC. Heat shock protein 60 is the major antigen which stimulates delayed-type hypersensitivity reaction in the macaque model of *Chlamydia trachomatis* salpingitis. Infect Immun **2004**; 72(2):1159–1161.

58. Kimani J, Maclean IW, Bwayo JJ, et al. Risk factors for *Chlamydia trachomatis* pelvic inflammatory disease among sex workers in Nairobi, Kenya. J Infect Dis **1996**; 173(6):1437–1444.

59. Hillis SD, Owens LM, Marchbanks PA, Amsterdam LE, Mac Kenzie WR. Recurrent chlamydial infections increase the risks of hospitalization for ectopic pregnancy and pelvic inflammatory disease. Am J Obstet Gynecol **1997**; 176:103–107.

60. Bakken IJ, Skjeldestad FE, Lydersen S, Nordbo SA. Births and ectopic pregnancies in a large cohort of women tested for *Chlamydia trachomatis*. Sex Transm Dis **2007**; 34(10):739–743.

61. Rank RG, Batteiger BE, Soderberg LS. Susceptibility to reinfection after a primary chlamydial genital infection. Infect Immun **1988**; 56(9): 2243–2249.

62. Rank RG, Soderberg LS, Sanders MM, Batteiger BE. Role of cell-mediated immunity in the resolution of secondary chlamydial genital infection in guinea pigs infected with the agent of guinea pig inclusion conjunctivitis. Infect Immun **1989**; 57(3):706–710.

63. Batteiger BE, Rank RG. Analysis of the humoral immune response to chlamydial genital infection in guinea pigs. Infect Immun **1987**; 55(8): 1767–1773.

64. Rank RG, Bowlin AK, Kelly KA. Characterization of lymphocyte response in the female genital tract during ascending Chlamydial genital infection in the guinea pig model. Infect Immun **2000**; 68(9): 5293–5298.

65. Debattista J, Timms P, Allan J, Allan J. Reduced levels of gamma-interferon secretion in response to chlamydial 60 kDa heat shock protein amongst women with pelvic inflammatory disease and a history of repeated *Chlamydia trachomatis* infections. Immunol Lett **2002**; 81(3):205–210.

66. Kinnunen A, Surcel H, Lehtinen M, et al. HLA DQ alleles and interleukin-10 polymorphism associated with *Chlamydia trachomatis*–related tubal factor infertility: a case-control study. Hum Reprod **2002**; 17(8):2073–2078.

67. Agrawal T, Gupta R, Dutta R et al. Protective or pathogenic immune

response to genital chlamydial infection in women—a possible role of cytokine secretion profile of cervical mucosal cells. Clin Immunol **2009**; 130:347–354.

68. Barr EL, Ouburg S, Igietseme JU, et al. Host inflammatory response and development of complications of *Chlamydia trachomatis* genital infection in CCR5-deficient mice and subfertile women with the CCR5delta32 gene deletion. J Microbiol Immunol Infect **2005**; 38(4): 244–254.

69. Dubin PJ, Kolls JK. IL-23 mediates inflammatory responses to mucoid *Pseudomonas aeruginosa* lung infection in mice. Am J Physiol Lung Cell Mol Physiol **2007**; 292(2):L519-L528.

70. Koenders MI, Kolls JK, Oppers-Walgreen B, et al. Interleukin-17 receptor deficiency results in impaired synovial expression of interleukin-1 and matrix metalloproteinases 3, 9, and 13 and prevents cartilage destruction during chronic reactivated streptococcal cell wall-induced arthritis. Arthritis Rheum **2005**; 52(10):3239–3247.

71. Kolls JK, Linden A. Interleukin-17 family members and inflammation. Immunity **2004**; 21(4):467–476.

72. Cruz A, Khader SA, Torrado E, et al. Cutting edge: IFN-gamma regulates the induction and expansion of IL-17-producing CD4 T cells during mycobacterial infection. J Immunol **2006**; 177(3):1416–1420.

73. Benwell RK, Lee DR. Essential and synergistic roles of IL1 and IL6 in human Th17 differentiation directed by TLR ligand-activated dendritic cells. Clin Immunol **2010**; 134:178–187.

74. Morre SA, Murillo LS, Bruggeman CA, Pena AS. The role that the functional Asp299Gly polymorphism in the toll-like receptor-4 gene plays in susceptibility to *Chlamydia trachomatis*–associated tubal infertility. J Infect Dis **2003**; 187(2):341–342.

75. Ouburg S, Spaargaren J, den Hartog JE, et al. The CD14 functional gene polymorphism −260 C>T is not involved in either the susceptibility to *Chlamydia trachomatis* infection or the development of tubal pathology. BMC Infect Dis **2005**; 5:114.

76. Murillo LS, Land JA, Pleijster J, Bruggeman CA, Pena AS, Morre SA. Interleukin-1B (IL-1B) and interleukin-1 receptor antagonist (IL-1RN) gene polymorphisms are not associated with tubal pathology and *Chlamydia trachomatis*-related tubal factor subfertility. Hum Reprod **2003**; 18(11):2309–2314.

77. Sziller I, Babula O, Ujhazy A, et al. *Chlamydia trachomatis* infection, Fallopian tube damage and a mannose-binding lectin codon 54 gene polymorphism. Hum Reprod **2007**; 22(7):1861–1865.

78. Swanson AF, Ezekowitz RA, Lee A, Kuo CC. Human mannose-binding protein inhibits infection of HeLa cells by *Chlamydia trachomatis*. Infect Immun **1998**; 66(4):1607–1612.

79. Peipert JF, Boardman L, Hogan JW, Sung J, Mayer KH. Laboratory evaluation of acute upper genital tract infection. Obstet Gynecol **1996**; 87:730–736.

80. Peeling RW, Kimani J, Plummer F, et al. Antibody to chlamydial hsp60 predicts an increased risk for chlamydial pelvic inflammatory disease. J Infect Dis **1997**; 175(5):1153–1158.

81. Peeling RW, Patton DL, Cosgrove Sweeney YT et al. Antibody response to the chlamydial heat-shock protein 60 in an experimental model of chronic pelvic inflammatory disease in monkeys (Macaca nemestrina). J Infect Dis **1999**; 180(3):774–779.

82. Pal S, Peterson EM, De La Maza LM. Vaccination with the *Chlamydia trachomatis* major outer membrane protein can elicit an immune response as protective as that resulting from inoculation with live bacteria. Infect Immun **2005**; 73(12):8153–8160.

83. Murthy AK, Chambers JP, Meier PA, Zhong G, Arulanandam BP. Intranasal vaccination with a secreted chlamydial protein enhances resolution of genital *Chlamydia muridarum* infection, protects against oviduct pathology, and is highly dependent upon endogenous gamma interferon production. Infect Immun **2007**; 75(2):666–676.