Gamma Interferon Is Required for Optimal Antibody-Mediated Immunity against Genital Chlamydia Infection

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Defining the mechanisms of immunity conferred by the combination of antibody and CD4\(^+\) T cells is fundamental to designing an efficacious chlamydial vaccine. Using the _Chlamydia muridarum_ genital infection model of mice, which replicates many features of human _C. trachomatis_ infection and avoids the characteristic low virulence of _C. trachomatis_ in the mouse, we previously demonstrated a significant role for antibody in immunity to chlamydial infection. We found that antibody alone was not protective. Instead, protection appeared to be conferred through an undefined antibody-cell interaction. Using gene knockout approaches, we demonstrated a significant role for antibody in immunity to chlamydial infection. We found that antibody alone was not efficacious as a chlamydial vaccine. Using the mouse genital infection model, we demonstrate a key function for IFN-\(\gamma\), which is responsible for more than 1.4 million genital infections annually worldwide (1). Although _C. trachomatis_ infection is the most common bacterial sexually transmitted infection (STI), there is currently no vaccine to aid in disease prevention. Genital infections in women are typically asymptomatic and can lead to severe pathology, including pelvic inflammatory disease (PID) and tubal factor infertility. With a great need for the development of an efficacious vaccine, it is important to understand the natural protective immunity that arises following genital infection with _Chlamydia_. The _C. muridarum_ model of murine genital tract infection has proven very useful for such studies as this model closely recapitulates the disease state observed in women, including ascension of _Chlamydia_ from the lower to the upper genital epithelium and irreversible pathological sequelae.

Adaptive immune responses are required to resolve _C. muridarum_ genital infection and to protect against reinfection. Athymic mice, major histocompatibility class (MHC) II knockout mice, T cell receptor (TCR) \(\alpha\beta\) knockout mice, and mice depleted of CD4\(^+\) T cells are unable to resolve primary genital chlamydia infection (2–5). Among the cellular responses, CD4\(^+\) T cells appear to be primary effectors in the resolution of genital infection. While the mechanisms for CD4\(^+\) T cell-mediated protection are not fully defined (6), some evidence has suggested that immunity provided by CD4\(^+\) T cells occurs through Plac8-mediated T cell degranulation (7) and gamma interferon (IFN-\(\gamma\)) production (4, 8–10).

The humoral arm of the adaptive immune response also contributes very importantly to chlamydial immunity. Antibody speeds the resolution of primary infection, provides a level of protection to reinfection that is equivalent to that of CD4\(^+\) T cells (5, 11, 12), and has been implicated in a protective role in vaccine studies (13, 14). Interestingly, CD4\(^+\) T cells appear to be playing a dual role in the protective response against _C. muridarum_, as they not only function to resolve primary infection independently of CD8\(^+\) T cells and antibody (12) but also function cooperatively with antibody (5, 6, 12). Several lines of evidence support the idea of a cooperative function between antibody and CD4\(^+\) T cells and provide evidence that CD4\(^+\) T cells are responsible for the recruitment and/or activation of a local effector cell population that functions with antibody to resolve infection. For example, the passive transfer of convalescent (immune) serum to naive C57BL/6 mice depleted of CD4\(^+\) T cells fails to protect the mice against primary _Chlamydia_ genital infection (mice are unable to resolve infection and shed very high numbers of bacteria). However, administration of that same serum to CD4\(^+\) T cell-sufficient naive mice at the time of primary infection decreases the infectious burden and shortens the course of infection compared to mice that do not receive immune serum (12). The protection afforded to naive mice by immune serum is particularly notable at days 10 to 14 of the infection, a time when the numbers of CD4\(^+\) T cells in the genital tract tissue are increasing (12). Lastly, antibody imparts a striking level of protective immunity against reinfection in the absence of CD4\(^+\) T cells (11). Collectively, these results strongly support the notion that the protective mechanism for antibody in chlamydial immunity is not direct bacterial neutralization (blocking of attachment) per se but that protection is instead mediated through the interaction of antibody with a yet-to-be-identified effector cell population.
Knowing that antibody alone does not protect a naïve genital tract from chlamydial infection and is protective only following CD4⁺ T cell priming (11,12), we reasoned that CD4⁺ T cell priming of the genital tract led to the recruitment and/or activation of an immune effector cell that interacts with antibody to confer protection. CD4⁺ T cells are known to secrete a variety of proinflammatory cytokines and chemokines that are capable of recruiting and activating other immune cells at the site of infection (4). One such cytokine that is produced in response to chlamydial genital infection is IFN-γ (4). Because IFN-γ is produced by CD4⁺ T cells and is known to activate cell populations that interact with antibody (e.g., macrophages) to kill pathogens, we sought to investigate the role of IFN-γ in antibody-mediated immunity to genital chlamydia.

In this study, we showed that the CD4⁺ T cell-secreted IFN-γ cytokine was necessary for optimal antibody-mediated immunity. The impact of IFN-γ on the protective response did not appear to be due to the recruitment of an effector cell population or to a change in the antibody response. Rather, our data support the hypothesis that CD4⁺ T cells cooperate with antibody in providing a striking level of protective immunity through the activation of an effector population that interacts with antibody to eliminate infectious chlamydiae.

**MATERIALS AND METHODS**

**Mice.** Female, 6-to-8-week-old wild-type C57BL/6, µMT (antibody-deficient) (B6.129P2-Ifg-6tm1Cgr/J), and IFN-γ⁻/⁻ (B6.129P2-IFn-g⁻/⁻) mice were purchased from Jackson Laboratory and housed in the animal facilities at the University of Arkansas for Medical Sciences (Little Rock, AR). Animal care and use protocols were approved by Institutional Animal Care and Use Committee and followed institutional guidelines.

**Chlamydia growth and purification.** C. muridarum (Weiss strain) was propagated in HeLa 229 cells, and infectious elementary bodies were harvested by density gradient purification as previously described (15).

**Genital infection and quantitation of chlamydiae.** Mice were infected subcutaneously with 2.5 mg of medroxyprogesterone acetate (Depo-provera) (Greenstone LLC) 10 and 3 days prior to primary infection and 5 days prior to secondary infection to synchronize the estrous cycles of the experimental mice. Prior to infection, the vaginal vault was gently swabbed with a Calgiswab (Puritan) to remove any mucus plugs.

To assess bacterial infection, cervicovaginal swabs were collected on days 3, 7, 10, and 14 and then every 7 days postinfection until infection resolved. Swabs were placed in 2-ml tubes with 0.5 ml SPG and 2 sterile 4-mm-diameter borosilicate glass beads (Fisher). Tubes were subjected to 20 strokes of a vortex mixer for 2 min at 4°C, 1,400 rpm using a Thermomixer R incubator (Eppendorf). Swabs were removed, an additional 0.5 ml of SPG was added to each tube to reach a total volume of 1 ml, and samples were stored at −80°C. Bacterial load was assessed by enumeration of IFUs on HeLa 229 monolayers (3). HeLa 229 cells were seeded at 2 X 10⁵ cells/well in 48-well cell culture plates. The following day, monolayers were washed with Hank’s balanced salt solution (HBBS) and treated with HBBS containing 45 µg/ml DEAE dextran for 10 min at room temperature. Cells were washed once with HBBS and infected with 300 µl of vaginal swab collections. Plates were centrifuged for 1 h at 37°C and 800 × g and then rested at 37°C for 30 min. Monolayers were then washed once with HBBS to remove unattached chlamydiae, and 0.5 ml Dulbecco’s modified Eagle medium containing 10% fetal bovine serum (DMEM-10), 10 µg/ml gen-
Quantitative real time-PCR (qRT-PCR) gene standards. C57BL/6 arginase I (Arg1) and inducible nitric oxide synthase (iNOS) genes were accessed via GenBank accession no. NM_007482.3 and NM_010927.4, respectively. Based on the predicted TaqMan primer/probe assay (Applied Biosystems) locations given by the manufacturer, a fusion was synthesized with Arg1 bp 54 to 331, linked to iNOS bp 2648 to 2924. A NcoI site was added to the start of Arg1, and overlapping BamHI (GGATCC) and Ncol (CCATGG) sites were added to the end of NO2. This fusion was created and cloned in pUC57 by GenScript. The lyophilized construct was reconstituted and transformed into TOP10 Escherichia coli for amplification. Cells were lysed, and amplified DNA was digested and gel purified. Total DNA was quantitated to calculate copy numbers appropriate for the qRT-PCR standard curve.

RNA isolation and qRT-PCR. Whole genital tracts were placed into M-tubes (Miltenyi Biotec) containing 0.5 ml to 1 ml TRIzol (Invitrogen) per 50 mg to 100 mg of tissue. Samples were homogenized with a gentleMACS Dissociator (Miltenyi Biotec) using the RNA_01 program. RNA was isolated using a RNasy Midi kit (Qiagen). cDNA was obtained with aSuperScript III first-strand synthesis system using random hexamers (Invitrogen). A 1-μg volume of total RNA was used for qRT-PCR on a StepOnePlus real-time system (Applied Biosystems). Samples were tested in triplicate and amplified using TaqMan primers and probes and TaqMan Advanced Fast master mix according to the instructions of the manufacturer (Applied Biosystems). For absolute transcript number calculations, RNA levels were determined by standard curve. For comparisons using the threshold cycle (2^(-△△CT)) method, fold change of infected to uninfected mice was determined after normalization to the HPRT1 housekeeping gene (Applied Biosystems).

Statistical analysis. Differences in IFU (infection) levels between treatments and strains of mice were determined using two-way analysis of variance (ANOVA) with the Bonferroni posttest. One-way ANOVA was used with the Bonferroni posttest to determine differences in antibody titers and qRT-PCR absolute transcript numbers. Student’s unpaired t-test was used to determine differences in qRT-PCR fold change analysis between wild-type and anti-CD4-treated mice within the individual time points tested. Statistical calculations were done using Prism 6.0f.

RESULTS

Effect of CD4+ T cell depletion on the cellular composition of genital tract tissue. Previous studies have shown that antibody-mediated protection against genital chlamydia infection is dependent on activation and/or recruitment of an effector cell population to the local genital tract tissues by CD4+ T cells. Once CD4+ T cells have primed the genital tract tissues, they can be eliminated, as they are no longer necessary for the immune protection provided by antibody (12). To determine if the role of CD4+ T cells in antibody-mediated protection was to recruit an effector cell population, we assessed whether immune cell emigration to the genital tract was affected by the depletion of CD4+ T cells.

Whole genital tracts from naïve C57BL/6 mice and from CD4-depleted and nondepleted mice were harvested at 7 and 21 days following primary infection and processed for flow cytometry analysis (Fig. 1). These time points following primary infection were chosen because CD4+ T cell priming of the genital tract tissues occurs during primary infection. Early in the course of primary infection (day 7), comparable levels of CD8+ T cells, dendritic cells, B cells, NK cells, macrophages, and neutrophils were present in CD4-depleted and nondepleted mice (Fig. 1B). By day 21 of infection, comparable levels of all cell types were found, with the exception of a larger number of CD8+ T cells in depleted mice (Fig. 1C), which are inconsequential in protective immunity to chlamydial genital infection (3, 5, 12). Overall, all immune cell populations were overwhelmingly similar regardless of CD4+ T cell depletion. Additionally, Fc receptor (FcR)-bearing cell populations (e.g., neutrophils, macrophages, NK cells, dendritic cells), presumably those that could function with antibody in protective immunity, were unaffected by CD4 depletion. Therefore, CD4+ T cell priming of the genital tract for antibody-mediated protective immunity against C. muridarum appears to be unrelated to the recruitment of immune cell populations.

IFN-γ is required for optimal antibody-mediated protection against infection. Since the recruitment of possible effector cells for antibody-mediated immunity was not impacted by CD4+ T cell depletion, we next assessed if CD4+ T cells were responsible for the activation of an effector cell population that functioned with antibody to resolve infection. Specifically, we chose to investigate the role of IFN-γ, the highly activating CD4+ T cell product. To begin to assess the contribution of IFN-γ to antibody-mediated immunity, we utilized IFN-γ−/− mice, along with C57BL/6 and μMT (antibody-deficient) control mice. The experimental design is schematically depicted in Fig. 2A and is based upon our previous experiments showing the protective contribution of antibody to chlamydial reinfection. One experimental difference from our previous studies, however, is that mice were treated with doxycycline following primary infection to ensure infection resolution before rechallenge. Antibiotic treatment is necessary because IFN-γ−/− mice are unable to completely resolve chlamydial infection (4, 17). To confirm that the treatment/rest regimen did not have any residual effects that could alter the course of infection, naïve C57BL/6 mice were treated with doxycycline every day for 2 weeks as described in Materials and Methods and were then rested for 10 days before primary infection. Doxycycline-treated mice showed no residual inhibitory effect of the antibiotic on the course of primary infection (Fig. 2B). Therefore, the treatment/rest regimen did not affect assessment of protective immunity in antibiotic-treated mice.

To determine the role of IFN-γ in antibody-mediated immunity to genital chlamydia infection, wild-type C57BL/6, μMT, and IFN-γ−/− mice were again used. Primary genital infection had completely resolved in C57BL/6 and μMT mice by 42 days postinfection (Fig. 3A) (5, 11, 12, 18). The resolution of primary infection in IFN-γ−/− mice was remarkably similar to that in C57BL/6 mice and μMT mice for the first few weeks, but IFN-γ−/− mice were unable to completely resolve genital infection (Fig. 3A) (4, 17). However, all strains of mice were culture negative following doxycycline treatment and prior to the initiation of secondary reinfection studies.

To assess efficacy of the antibody response specifically, mice were depleted of CD4+ cells and rechallenged with C. muridarum. All strains sufficient in CD4+ T cells were markedly protected against reinfection (Fig. 3B) (17). Similarly, wild-type mice depleted of CD4+ T cells were also protected due a functional antibody response (Fig. 3C). The severely immunocompromised CD4-depleted μMT mice were unable to resolve infection (5, 12) (Fig. 3C). Secondary infection resolved in CD4-depleted μMT mice only after anti-CD4 treatment had ceased and CD4+ T cells repopulated in each animal. Interestingly, CD4-depleted IFN-γ−/− mice displayed an intermediate level of protective immunity to reinfection, characterized by the shedding of large numbers of infectious chlamydiae and an infection course of much longer duration compared to those seen with C57BL/6 controls (Fig. 3C). However, infection did begin to resolve during the time CD4+ T cells were functionally depleted (i.e., day 21). Together, these data...
demonstrate that IFN-γ plays a key role for optimal antibody-mediated immunity to *Chlamydia* genital reinfection, although other factors may also contribute.

**Chlamydia-specific antibody responses in IFN-γ−/− mice.** IFN-γ is known to play a role in antibody production and isotype switching in response to an infection (19–22), suggesting that the diminished antibody-mediated protection observed during secondary infection in CD4-depleted IFN-γ−/− mice could be due to a different, less protective antibody response in the absence of IFN-γ. To determine if the antibody response of IFN-γ−/− mice differed from the antibody response of wild-type C57BL/6 mice, serum was collected during primary infection, before CD4− T cell depletion, and during secondary infection. ELISA analysis determined that, while the IgG2c response was somewhat delayed during primary infection in the IFN-γ−/− mice, C57BL/6 and IFN-γ−/− mice had comparable immunoglobulin class and subclass chlamydia-specific antibody titers prior to secondary infection (Fig. 4A and B). Serum antibody titers during secondary infection confirmed that these responses were maintained during infection and that CD4+ T cell depletion prior to reinfection had little to no effect on the magnitude of the antibody response (Fig. 4C). Therefore, the decrease in protection seen in CD4-depleted IFN-γ−/− mice was not due to differences in the chlamydia-specific antibody response.

**Immune cell genital tract infiltration following infection in CD4-depleted IFN-γ−/− mice.** Although immune populations emigrate normally to the genital tract following CD4− T cell depletion in C57BL/6 mice (Fig. 1), IFN-γ has been shown to recruit neutrophils and other cell populations to sites of infection (23). In order to rule out the possibility of a defect in IFN-γ-mediated...
recruitment of an effector population in CD4-depleted IFN-γ−/− mice, IFN-γ−/− and μMT control mice were depleted of CD4+ T cells during primary infection (as shown in Fig. 1) and levels of immune cell populations present in the genital tract were determined by flow cytometry. Overall, CD4+ T cell depletion did not affect the recruitment of major immune populations to the genital tract at any time point during primary chlamydial infection in IFN-γ−/− or μMT mice (Fig. 5). Thus, the diminished protection seen in CD4-depleted IFN-γ−/− mice was not due to a defect in CD4-mediated recruitment of an effector population.

Effect of CD4+ T cell depletion on the global activation state of infected genital tracts. IFN-γ is a potent activator of many cellular immune processes in response to infection. The necessity of IFN-γ for optimal antibody-mediated protection against reinfection led us to ask if we could established global differences in the immune activation status of the genital tract during C. muridarum infection in the presence and absence of CD4+ T cells. Specifically, since macrophages and neutrophils are effector cells that interact with antibody and can be activated by IFN-γ, we evaluated the effect of CD4+ T cell depletion on various macrophage and neutrophil activation markers. To determine the type and magnitude of immune activation in the genital tract, we measured mRNA levels of downstream products of IFN-γ activation, as well as other markers representing a spectrum of inflammation states, including inducible nitric oxide synthase, arginase 1 (Arg1), Ym1, Fizz1, interleukin 10 (IL-10), and interleukin 6 (IL-6). To determine the specific effect of CD4+ T cells on activation, C57BL/6 mice were depleted of CD4+ T cells during primary C. muridarum infection and whole genital tract qRT-PCR was performed on days 7 and 14, comparing genital tract mRNA levels in CD4-depleted mice to the levels in the non-CD4-depleted controls. iNOS transcript levels were greatly increased over the levels seen with naive mice during infection as expected, but similar transcript numbers were seen in CD4-depleted and nondepleted mice at days 7 and 14 (Fig. 6A). Similar levels of IL-10, Ym1, Fizz1, IL-6, and tumor necrosis factor alpha (TNF-α) were also observed regardless of CD4+ T cell depletion status (Fig. 6B).

DISCUSSION

There is a great need for a vaccine to aid in prevention of chlamydial infection on a global scale. While previous studies have shown that both CD4+ T cells (2–5) and antibody (5, 11, 12) play central roles in protection against C. muridarum infection, we sought in this study to uncover the mechanism(s) by which antibody mediates immunity. Delineation of specific mechanisms of protection will greatly facilitate the development of an efficacious vaccine. Previous studies have shown that antibody provides a striking level of protective immunity to chlamydial genital infection (5, 11, 12) and that antibody-mediated protection is dependent on CD4+ T cells orchestrating the recruitment and/or activation of an effector cell population (12), since antibody does not appear to protect in vivo via direct chlamydial neutralization (12, 24). While IFN-γ has been shown to be directly and indirectly involved in the recruitment of neutrophils and inflammatory macrophages (two major FcR-bearing populations present during chlamydial infection) in a variety of infection settings (25–28), we showed in the current study that immune cell recruitment is not negatively impacted by the absence of IFN-γ during C. muridarum genital infection. Importantly, several additional cytokines and chemokines secreted by CD4+ T cells, including IL-17 and IL-6, are proinflammatory and capable of recruiting immune cells to the genital tract during infection (29, 30). We found that, similarly to IFN-γ−/− mice, CD4 depletion had minimal impact on overall immune cell infiltration during infection in all mouse strains tested, with the exception of an increase in CD8+ T cell levels at day 21 postinfection. However, this increase in CD8+ T cell levels does not equate with protection, as CD4+ T cell-depleted CD8+ T cell-sufficient mice are incapable of resolving C. muridarum infection or priming the genital tract for antibody-mediated immunity (3, 5, 12). The similarities in cellular infiltration between CD4-depleted and nondepleted mice strongly support the notion that IFN-γ-mediated effector cell activation, rather than effector cell recruitment, is central to optimal antibody-mediated immunity.

It is generally accepted that there are both IFN-γ-dependent and -independent mechanisms that aid in the clearance of primary genital C. muridarum infection (4, 17). IFN-γ is secreted in large quantities by CD4+ T cells during chlamydial infection and is known to play a role in immunoglobulin class switching. B cell maturation, and activation of several immune cell populations and processes during bacterial infections (19, 31–37). In our current study, however, we identify a new role for IFN-γ in the pro-
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During primary and secondary infections, the chlamydia-specific antibody responses were largely similar in magnitude and class/subclass specificity in C57BL/6 and IFN-γ/H9253/H11002 mice, suggesting that the prominent role for IFN-γ is independent of class switching (Fig. 4) (35). Moreover, it was expected on the basis of previous studies (4, 17) that IFN-γ would play an important role in the cellular T_h1 response to genital chlamydia infection, but its necessity for antibody-mediated immunity (not antibody production) in chlamydial genital infection shows an additional functional role of this cytokine in chlamydial immunity.

IFN-γ was required for optimal antibody-mediated protection. Infection resolution began prior to the end of CD4+ T cell depletion in IFN-γ−/− mice (Fig. 3C), indicating that other mediators must be involved in addition to IFN-γ. CD4+ T cells secrete a plethora of activating cytokines during C. muridarum infection, including IL-17 (38, 39). IL-17 plays multiple roles, including recruiting and activating various immune cell populations at the local infection site, during infections by bacterial species such as Bordetella pertussis and Klebsiella pneumoniae (40–43). Furthermore, IL-17 is capable of acting both alone and together with IFN-γ in reducing chlamydial burden in epithelial cells and macrophages in vitro (44) and has also been shown to act synergistically with IFN-γ to kill pathogens in vivo (45–47). Therefore, because genital C. muridarum infection induces a T_h17 response and IL-17 activates phagocytic cells (38, 39, 44, 48, 49), it will be
important to further evaluate the contribution of IL-17 in antibody-mediated chlamydial immunity. The direct mechanism(s) of antibody-mediated protection has not been fully elucidated. However, previous work has suggested a variety of roles for antibody during chlamydial infection, including both indirect immune support such as enhancement of T cell activation by concentrating antigen within professional antigen-presenting cells (APCs) and facilitation of epithelial cell killing via antibody-dependent cell-mediated cytotoxicity (ADCC) by macrophages (50, 51). It has also been proposed that antibody plays a key role in preventing the dissemination of chlamydia to extragenital sites (52), which may or may not be related to the dissemination seen in IFN-$\gamma$-mediated activation event(s) required for antibody to protect during genital chlamydia infection, our data allude to an important role for the highly activating IFN-$\gamma$ in enhancing the antimicrobial activity of phagocytes and/or increasing the expression of FcRs to augment phagocytosis of antibody-coated chlamydiae, as has been shown for other bacteria (53–56). Further supporting this notion is the dramatic increase in the number of macrophages and neutrophils (both FcR-bearing phagocytic populations) in the genital tissue following infection with C. muridarum (Fig. 1 and 5). Additionally, for another intracellular bacterium, Francisella tularensis, phagocyte-dependent antibody-mediated protection is completely dependent upon the secretion of IFN-$\gamma$ by CD4$^+$ T cells (57). The concept of increased phagocyte activation and killing of antibody-opsonized intracellular pathogens is not new (58–62), but in the current study we have uncovered a possible unique mechanism by which the genital tract tissues are primed during primary chlamydial infection for subsequent protection to occur via phagocyte-antibody interactions. While we hypothesize that increased phagocytic killing is largely responsible for antibody-mediated protection, combinations of multiple mechanisms and mediators are likely required for complete immunity. The lack of differences seen in the global activation state of the infected genital tract with CD4$^+$ T cell depletion as measured by qRT-PCR (Fig. 6) may be due to other immune effectors playing compensatory roles to regulate the transcripts measured.

A direct role for antibody in mediating immunity against human genital chlamydia infection has not been firmly established, even though subjects with ongoing C. trachomatis genital infection produce significant chlamydia-specific antibody responses (63). While one study found an association between high IgA titers and bacterial clearance in the endocervix (64), most chlamydia serologic responses do not correlate with protective immunity. This lack of a strong correlation between antibodies and
protective immunity in humans is not unlike what was initially concluded from the murine model of *C. muridarum* genital infection. Specifically, mice develop striking protective immunity in the absence of antibody (18), which led to the notion that antibody was unimportant in chlamydial immunity. The considerable protective role of antibody in chlamydial immunity was not appreciated until the protective CD4$^+$ T cell responses were eliminated (5, 11, 12). Thus, perhaps the absence of obvious antibody-mediated protection in humans results from the inability to clearly delineate protective CD4$^+$ T cell responses and protective antibody responses. An alternative explanation for the absence of a strong correlation between antibody and protective immunity to human chlamydial genital infection comes from our previous work using the murine infection model (5, 11, 12, 14) and this current study. We have shown that antibody protects only following CD4$^+$ T cell priming of the genital tract tissues. Because the majority of human chlamydial genital infections are without signs or symptoms, suggesting mild inflammation, perhaps this mild inflammatory response results in incomplete or ineffective priming of the genital tract by CD4 T$^+$ cells during infection. Further studies will be needed to demonstrate whether equivalent immune mechanisms function to resolve human infection and to protect against reinfection.

Overall, our data provide important insight into the ability of antibody to protect after CD4$^+$ T cell priming of the genital tract during initial *Chlamydia* infection. We provide evidence that IFN-$\gamma$ is important for genital tract priming and that activation of an effector cell population(s) by IFN-$\gamma$ may be key to this response. Further elucidation of the mechanisms of protective adaptive immune responses, both cellular and humoral, during chlamydia infection will provide further insight into the type(s) of response(s) that needs to be elicited by a successful vaccine.
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