Murine *Chlamydia trachomatis* Genital Infection Is Unaltered by Depletion of CD4$^+$ T cells and Diminished Adaptive Immunity

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Chlamydia muridarum* and *Chlamydia trachomatis* mouse models of genital infection have been used to study chlamydial immunity and vaccine development. To assess the protective role of CD4$^+$ T cells in resolving *C. trachomatis* and *C. muridarum* genital tract infections, we used the female mouse model and evaluated infection in the presence and absence of CD4$^+$ T cells. In contrast to *C. muridarum* infection, *C. trachomatis* infection was unaltered in the absence of CD4$^+$ T cells. Mice infected with *C. trachomatis* developed protective immunity to re-challenge, but unlike *C. muridarum* infection, optimum resistance required multiple infectious challenges, despite the generation of adaptive serum and local chlamydial specific immune responses. Thus, understanding the chlamydial pathogenic and host immunologic factors that result in a diminished protective role for CD4$^+$ T cells in *C. trachomatis* murine infection might lead to new insights important to human immunity and vaccine development.

The obligate intracellular bacterial pathogen *Chlamydia trachomatis* causes an estimated 92 million new cases of sexually transmitted infection each year worldwide [1]. Lasting and durable control of these infections, and prevention of the deleterious consequences of infection, will require the development of a vaccine. A limitation to the development of a vaccine relates to the incomplete understanding of the natural history of human chlamydial genital infection. Diagnosis of infection mandates treatment, and thus it is not fully understood which responses are necessary for protective immunity and when during

the course of infection those responses mature and become effective. Therefore, *Chlamydia* infection of animals—primarily mice—has been used as a surrogate to study the natural history of genital infection and the development of adaptive immunity. Two mouse models of infection have been used extensively to study chlamydial genital infection and immunity: one model uses the mouse pathogen, *Chlamydia muridarum;* and the other uses the human pathogen, *C. trachomatis.* The need for adaptive immunity in resolving murine *C. muridarum* infection is unequivocal [2], but the role of adaptive immunity in resolving murine *C. trachomatis* infection is ambiguous [3, 4], even though primary infection elicits adaptive immune responses and a level of resistance to reinfection appears to develop [5–10].

In this study we sought to directly examine the contribution of adaptive immunity (CD4$^+$ T cells) in resolving primary murine *C. trachomatis* genital infection. Because previous studies have clearly demonstrated that *C. trachomatis* genital infection of mice is highly dependent on mouse strain [5, 11], it was crucial to choose a mouse strain that supported a robust infection that persisted for sufficient time to allow for the development of adaptive immune responses. Accordingly, we chose to use the

C3H/HeJ strain of mice. Mice of the C3H background have been shown to be susceptible to *C. trachomatis* genital infection, and the C3H/HeJ strain in particular has impaired innate immunity due to a nonfunctional Toll-like receptor (TLR) 4 [12]. Those combined attributes should favor the development of an infection of sufficient intensity and duration to delineate the role of adaptive immunity in resolving *C. trachomatis* genital infection. We show here that CD4$^+$ T cell–mediated adaptive immunity is not required to control primary murine *C. trachomatis* genital infection.

MATERIALS AND METHODS

Mice
Female C3H/HeJ mice were purchased from the Jackson Laboratories and maintained in the animal facilities at the University of Arkansas for Medical Sciences (Little Rock). Mice aged 6–10 weeks were used for these studies. All experimental procedures were performed in accordance with institutional policies for animal health and well being and were approved by the Institutional Animal Care and Use Committee.

Bacteria
C. muridarum (Weiss strain; formerly known as *C. trachomatis* strain mouse pneumonitis) and *C. trachomatis* serovars A (strain 2497) [13], D (strain UW-3/CX), and L2 (strain 434) were grown in HeLa 229 cells and purified by density gradient centrifugation [14].

Genital Infection and Enumeration of Chlamydia
Mice were infected and chlamydiae were enumerated as previously reported [15]. In brief, at 10 and 3 days prior to infectious challenge, mice were injected subcutaneously with 2.5 mg of medroxyprogesterone acetate (SICOR Pharmaceuticals). Before infectious challenge, vaginal vaults of progesterone treated mice were gently swabbed with a calcium alginate tipped swab (Fisher Scientific) to remove excess mucous. Mice were challenged by placing 5 μL containing either 5×10^4 inclusion forming units (IFUs) of *C. muridarum*, or 1×10^5 IFUs of *C. trachomatis* serovar A, D, or L2 into the vaginal vault. Genital infection was assessed by swabbing the vaginal vault (rotating the swab 8 times clockwise and 8 times counter-clockwise), placing the swab into a tube containing 1.0 mL of SPG (0.25 M sucrose/ 10 mmol/L sodium phosphate/5 mmol/L L-glutamic acid; pH, 7.2) and two 4-mm glass beads, shaking for 5 min, and then inoculating 0.3 mL of appropriately diluted samples into duplicate wells of a 48-well plate containing HeLa 229 cell monolayers. Plates were centrifuged at $780 \times g$ for 1 h at 37°C and rested for 30 min at 37°C, and the inoculum was removed and replaced with Dulbecco's Modified Eagles Medium containing 10% fetal bovine serum, 10 μg/mL gentamicin, and 1 μg/mL cycloheximide. After 30 h of incubation at 37°C, the supernatant was removed, cells were fixed with methanol, and the number of IFUs was visualized by indirect immunofluorescent staining using a chlamydial lipopolysaccharide mAb EVI-H1 [16].

Antibody Analysis
Serum and vaginal washes were collected and analyzed by enzyme-linked immunosorbent assay (ELISA) for *Chlamydia*-specific antibody, as described elsewhere [15]. All ELISAs were performed using elementary bodies (EBs) matched to the infecting *Chlamydia* strain (eg, serum and vaginal washes from serovar A–infected mice were analyzed using serovar A EBs) as ELISA antigen. Serum titers are expressed as the inverse of the highest serum dilution giving an absorbance (optical density at 405 nm [OD$_{405}$]) of $\geqslant 0.25$, which represents an OD$_{405}$ value that is 3 standard deviations greater than the mean OD$_{405}$ of preimmune serum. For vaginal washes, data are reported as the number of mice positive for *Chlamydia* antibody/ total number tested. Positive responses are defined as an OD$_{405}$ $\geqslant$ 0.1, which represents and OD$_{405}$ value that is 3 standard deviations greater than the mean OD$_{405}$ of preimmune vaginal washes.

CD4$^+$ T Cell Depletion
Mice were depleted of CD4$^+$ T cells as previously described [17, 18]. Four hundred micrograms of anti-CD4 mAb (clone GK1.5) was injected intraperitoneally according to the following schedule (day 0 represents day of infection): days -6, -5, -4, -1, 2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, and 41. Following this treatment regimen, CD4$^+$ T cells remain depleted throughout the test period, as noted by a >99% depletion of those cells by fluorescent-activated cell sorting analysis and greatly diminished antigen-specific antibody and delayed-type hypersitivity responses [17, 18]. We have found that, if the aforementioned treatment regimen is followed, mice can be functionally depleted of CD4$^+$ T cells (as assessed by greatly diminished antibody and cell-mediated immune (CMI) responses) for no longer than 50 days, after which time helper T cell functions return (antigen-specific antibody and CMI responses), even with continued anti-CD4 treatment. Therefore, all experiments using CD4-depleted mice are terminated 40–43 days after chlamydial infection.

Histological Analsysis
Mice were killed 7 and 14 days after primary infection. Genital tracts were removed and processed for histopathological analysis [15]. Thin sections were stained with hematoxylin and eosin and evaluated by a veterinary pathologist.

Statistical Analysis
Two-way analysis of variance with Bonferroni posttest was used to analyze the differences in IFU counts (infection) between groups. The Student *t* test was used to identify differences between serological responses.

RESULTS
Course of *C. muridarum* Genital Infection in C3H/HeJ (TLR4-Deficient) Mice
The primary objective of the study was to use the mouse model of *C. trachomatis* genital infection to assess the impact of CD4$^+$ T cell responses in resolving infection. Because previous studies

suggest that innate immunity alone can restrict the establishment of a robust *C. trachomatis* infection in some strains of mice [4], we selected the TLR4-deficient C3H/HeJ mouse strain to use in our studies. By using C3H/HeJ mice rather than the fully immunocompetent C3H/HeN strain of mice, we expected that the innate immune defect would facilitate infection, without negatively impacting the development of protective adaptive immunity. To show that the TLR4 defect did not negatively impact the development of protective adaptive immunity, we assessed infection and immunity to reinfection using the *C. muridarum* genital infection model. This infection model has clearly demonstrated the key role for adaptive immunity, and CD4+ T cells specifically, in infection resolution and immunity to reinfection [17, 18]. Genital challenge of C3H/HeJ mice with *C. muridarum* produced a robust infection, characterized by the shedding of $>10^6$ IFUs soon after challenge, with complete resolution by 5–7 weeks (Figure 1A and B). Mice that had resolved a primary genital infection were markedly protected from reinfection, as demonstrated by a significant reduction in bacterial shedding ($>4 \log_{10}$ IFU reduction; $P < .001$ at all time points) (Figure 1C) and shortened duration of infection (3–28 days) (Figure 1D). These results confirmed that the TLR4 defect did not negatively impact the course of infection or the development of adaptive immunity to reinfection.

Course of Primary, Secondary and Tertiary *C. trachomatis* Genital Infection in C3H/HeJ (TLR4-Deficient) Mice

Because the TLR4-deficient C3H/HeJ mouse strain proved to be a useful model for assessing adaptive immunity to chlamydial genital infection, we next evaluated the ability of C3H/HeJ mice to resolve primary *C. trachomatis* infection and to resist reinfection. Mice were challenged with *C. trachomatis* serovar A, D, or L2, and the course of infection was monitored by enumerating IFUs. As a group, primary genital infection with *C. trachomatis* serovar A, D, or L2 resulted in shedding of fewer chlamydiae, compared with infection with *C. muridarum* (approximately 2–4 $\log_{10}$ IFU lower; $P < .001$) (Figure 1A and Figure 2A). Mice challenged with serovar A shed fewer chlamydiae than those challenged with either serovar D or L2 (Figure 2A; see legend for P values), and a large proportion of serovar A–infected mice resolved the infection more quickly than mice infected with serovar D or L2 ($P < .05$) (Figure 2B). Histological evaluation of genital tracts 7 and 14 days after primary infection revealed minimal inflammation and no evidence of lymphocyte clusters (data not shown), which was not unlike that which has been reported previously [19].

Significant protection from reinfection was observed when mice were rechallenged with the homologous *C. trachomatis* serovar (Figure 2). This was particularly evident when comparing
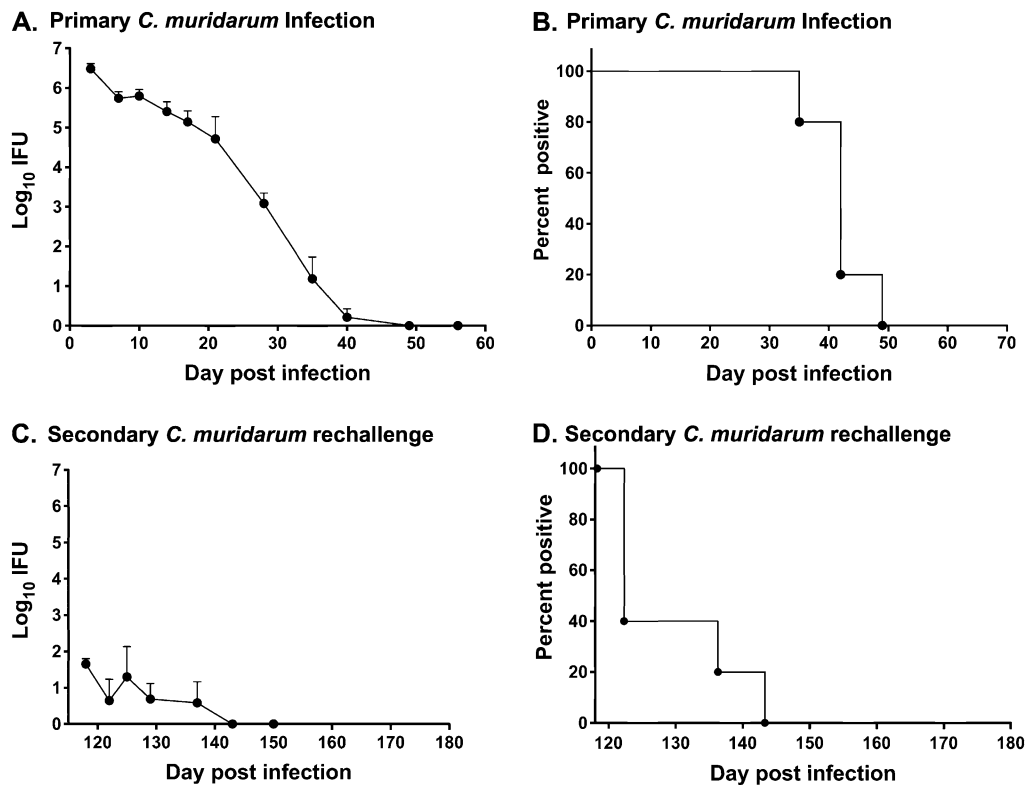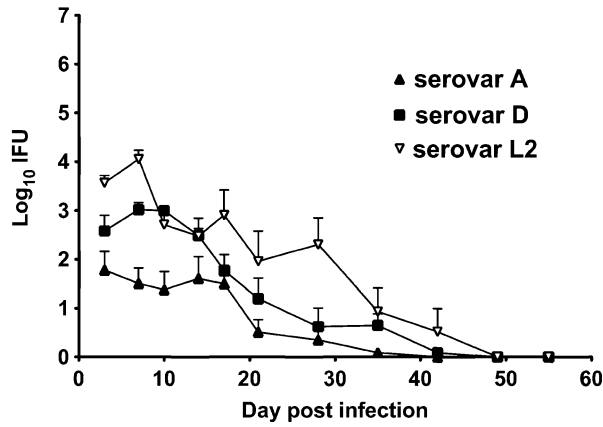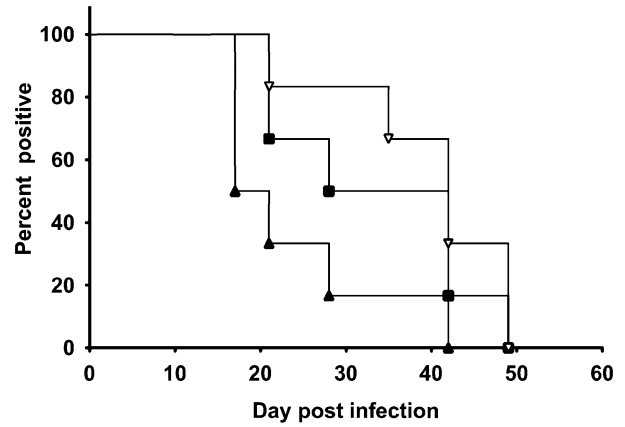

Figure 1. Course of primary *(A and B)* and secondary *(C and D) Chlamydia muridarum* infection in female C3H/HeJ mice. Medroxyprogesterone acetate–treated mice were challenged vaginally with *C. muridarum,* and the course of infection was monitored by enumerating chlamydiae recovered from cervicovaginal swabs collected at various times following challenge. Data are presented as mean inclusion forming units (IFUs) ± standard error of the mean for 6 mice *(A and C)* and as the percentage of culture-positive animals *(B and D).* "Day post infection" refers to the day after primary infectious challenge.
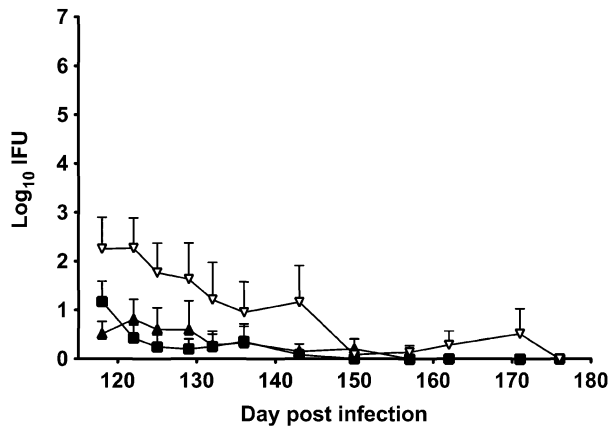
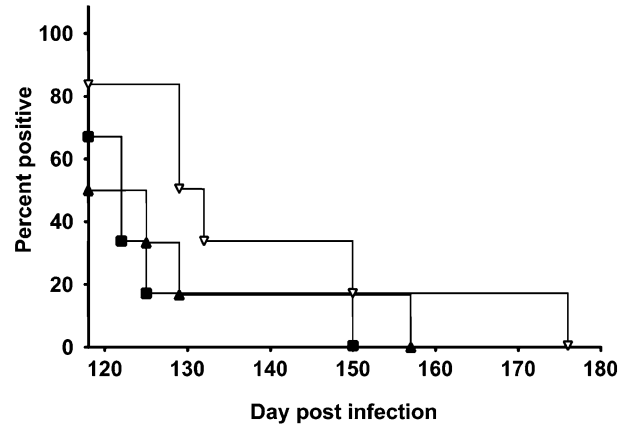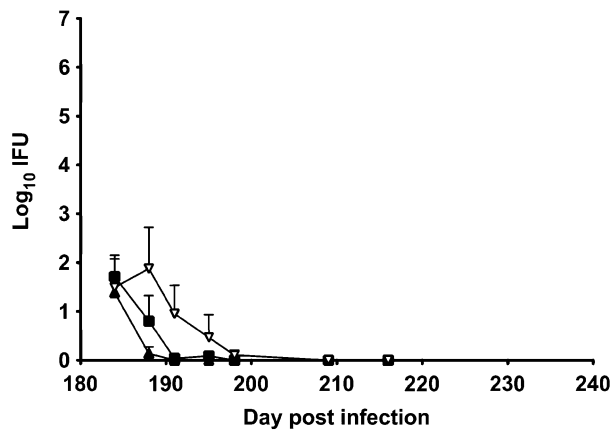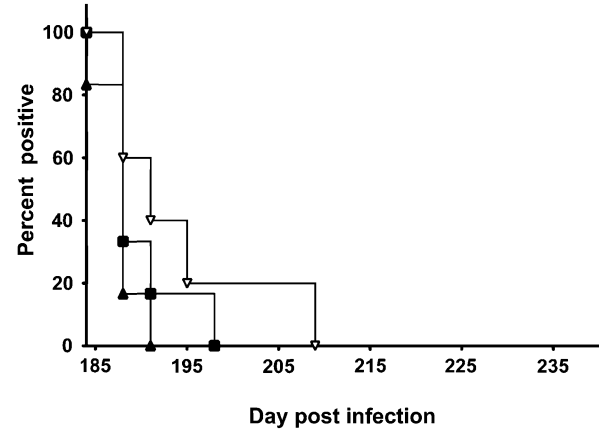**Figure 2.** Course of primary *Chlamydia trachomatis* genital infection *(A and B)*, and secondary *(C and D)*, and tertiary infectious challenge *(E and F)* in female C3H/HeJ mice. Medroxyprogesterone acetate–treated mice were challenged vaginally with either *C. trachomatis* serovar A (▲), D (■), or L2 (▽), and infection was monitored by enumerating chlamydiae recovered from cervicovaginal swabs. Data are presented as mean inclusion forming units (IFUs) ± standard error of the mean for 6 mice per group *(A, C, and E)* and as the percentage of culture-positive animals *(B, D, and F)*. "Day post infection" refers to the day following primary infectious challenge. For clarity, we have listed below the various statistical comparisons between the infection curves rather than on the figures. Primary infection curves: for A vs D, $P < .01$ for days 7 and 10; for A vs L2, $P < .001$ for days 3, 7, and 28, $P < .01$ for day 21, and $P < .05$ for days 10 and 17; for L2 vs D, $P < .01$ for day 28. Comparison of primary infection (1°) with secondary infection (2°) with the homologous serovar (eg, serovar A primary infections vs serovar A secondary infection): for 1° serovar A vs 2° serovar A, $P < .05$ for days 3 and 17; for 1° serovar D vs 2° serovar D, $P < .001$ for days 3, 7, 10, 14 and 17; for 1° serovar L2 vs 2° serovar L2, P was not significant at any time point. For comparison of primary infection vs tertiary infection with the homologous serovar for days 3, 7, 10, and 14 days post infection, $P < 0.01$ for all serovars.

primary infection to tertiary infection (eg, primary serovar A infection versus tertiary infection with serovar A) (Figure 2A, 2C, and 2E), although some protection (lower bacterial shedding) was observed on specific days post-secondary infection (see Figure 2 legend for *P* values). Thus, vaginal challenge of female C3H/HeJ mice with *C. trachomatis* (serovars A, D, or L2) results in an infection having a similar duration to that observed with *C. muridarum* infection, but far fewer bacteria are shed throughout the course of infection. Furthermore, the level of immunity elicited following primary *C. trachomatis* infection is far less than that elicited following *C. muridarum* infection. A single *C. muridarum* infection results in marked protective immunity, whereas 2 infections are necessary to stimulate significant immunity in the *C. trachomatis* infection model.

Characteristics of the *Chlamydia*-Specific Antibody Response

Serum and vaginal washes were collected and analyzed by ELISA using EBs matched to the infecting serovar as antigen. Although there were some differences in the magnitude of the responses among and between the serovars, compared with *C. muridarum*, in general the responses were remarkably similar. All infections resulted in high-titer immunoglobulin (Ig) G2a after primary infection, and the magnitude of those responses remained unchanged following secondary and tertiary infection (Figure 3B,

3C, and 3D). The IgG1, IgG2b, and IgG3 responses were somewhat more variable during primary infection, with lower titer responses in mice infected with either serovar A or L2. Overall, the antibody responses elicited by infection with *C. trachomatis* serovars and *C. muridarum* were consistent, with the exception of the serum IgA response. Notably, infection with *C. trachomatis* serovar A or L2 failed to elicit detectable serum anti-*Chlamydia* IgA even after 3 infectious challenges, and infection with serovar D produced a very unremarkable IgA response after primary infection, and this response did not increase in magnitude upon multiple reinfections. In contrast, *C. muridarum* infection elicited a substantial and long-lasting IgA response (Figure 3).

Vaginal washes were also analyzed for *Chlamydia*-specific antibody (Table 1). As shown previously [15], vaginal washes from mice infected with *C. muridarum* were positive for anti-chlamydial IgA early after infection (14 days), with IgG2a and IgG2b becoming positive later (28 days). Conversely, only a fraction of the *C. trachomatis*–infected mice had detectable anti-chlamydial IgA at 28 days after primary infection, and none were positive for IgG2a or IgG2b. After tertiary infection, nearly all *C. trachomatis*–infected mice had anti-chlamydial IgG2a in vaginal washes, and most were also positive for IgA. Thus, although *C. trachomatis* infection does not stimulate heightened
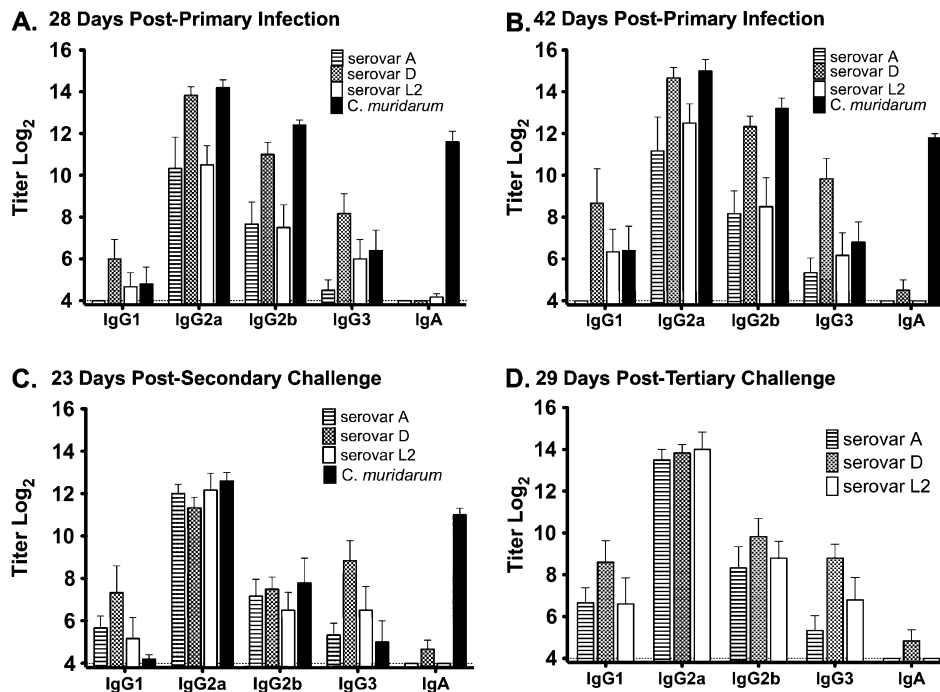
Figure 3. Immunoglobulin class and subclass specificity of the anti-*Chlamydia* antibody response. Serum samples collected from individual mice (6 per group) infected with *Chlamydia trachomatis* serovar A (▤), D (▨), or L2 (□) or with *Chlamydia muridarum* (■) were tested for anti-*Chlamydia* antibody using the homologous infecting *Chlamydia* strain as antigen in the enzyme-linked immunosorbent assay (eg, serum from serovar A–infected mice was tested against serovar A EBs). Data are presented as the mean anti-*Chlamydia* titer ± standard error of the mean for the indicated class and subclass immunoglobulins of serum collected 28 days after primary infection *(A)*, 42 days after primary infection *(B)*, 23 days after secondary challenge *(C)*, and 29 days after tertiary challenge *(D)*. Ig, immunoglobulin.

Table 1. Anti-*Chlamydia* Antibody Responses in Vaginal Washes

Mouse infection[a]	Antibody response (no. of animals positive/total no. tested)								
	14 days after primary challenge			28 days after primary challenge			49 days after tertiary challenge		
	IgG2a	IgG2b	IgA	IgG2a	IgG2b	IgA	IgG2a	IgG2b	IgA
Chlamydia trachomatis A	0/3	0/3	0/3	0/3	0/3	0/3	6/6	0/6	4/6
C. trachomatis D	0/4	0/4	0/4	0/4	0/4	2/4	6/6	3/6	5/6
C. trachomatis L2	0/3	0/3	0/3	0/3	0/3	1/3	5/6	0/6	2/6
Chlamydia muridarum	0/3	0/3	3/3	3/3	1/3	3/3	nt	nt	nt

NOTE. Vaginal washes were analyzed for antibody responses to EBs of the infecting chlamydial strain. Immunoglobulin (Ig) G1 and IgG3 responses are not included as vaginal washes are routinely negative for those antibody subclasses.

[a] Mice were infected with either *C. trachomatis* serovar A, D, or L2 or *C. muridarum*. To obtain an accurate depiction of vaginal wash antibodies, washes were obtained from independent groups of mice that had not been previously cultured, which is the basis for different group sizes for primary and tertiary infection time points.

systemic anti-chlamydial IgA responses, infection does result in the local production of antigen-specific IgA, albeit delayed.

The Role of CD4+ T Cells in Resolving *C. trachomatis* Genital Infection

To directly test the role of CD4+ T cell–mediated adaptive immunity in resolving primary *C. trachomatis* genital infection, the course of infection was monitored in CD4+ T cell–depleted and –nondepleted C3H/HeJ mice. Depletion of CD4+ T cells has previously been shown to inhibit chlamydiae-specific cell-mediated immune responses (delayed type hypersensitivity) and T cell–dependent antibody responses [18, 20]. Using specific antibody responses as a measure of adaptive immunity, we show that CD4+ T cell depletion significantly inhibited the production of chlamydiae-specific antibody responses (Figure 4). Although

Chlamydia-specific antibody responses began to develop by 42 days after infection (specifically in mice infected with *C. muridarum* and *C. trachomatis* serovar L2), those responses coincided with the diminished effectiveness of anti-CD4 depletion at this late time point.

Depletion of CD4+ T cells did not significantly impact ($P > .05$) the course of *C. trachomatis* serovar A (Figure 5A), D (Figure 5B), or L2 (Figure 5C) genital infection. At no time point was bacterial shedding from CD4-depleted mice significantly different ($P > .05$) from untreated mice, regardless of the infecting serovar. Conversely, depletion of CD4+ T cells markedly impacted the ability of mice to resolve *C. muridarum* genital infection (Figure 5D) [17]. CD4-depleted *C. muridarum*–infected mice remain infected during the period of depletion and shed significantly greater numbers of
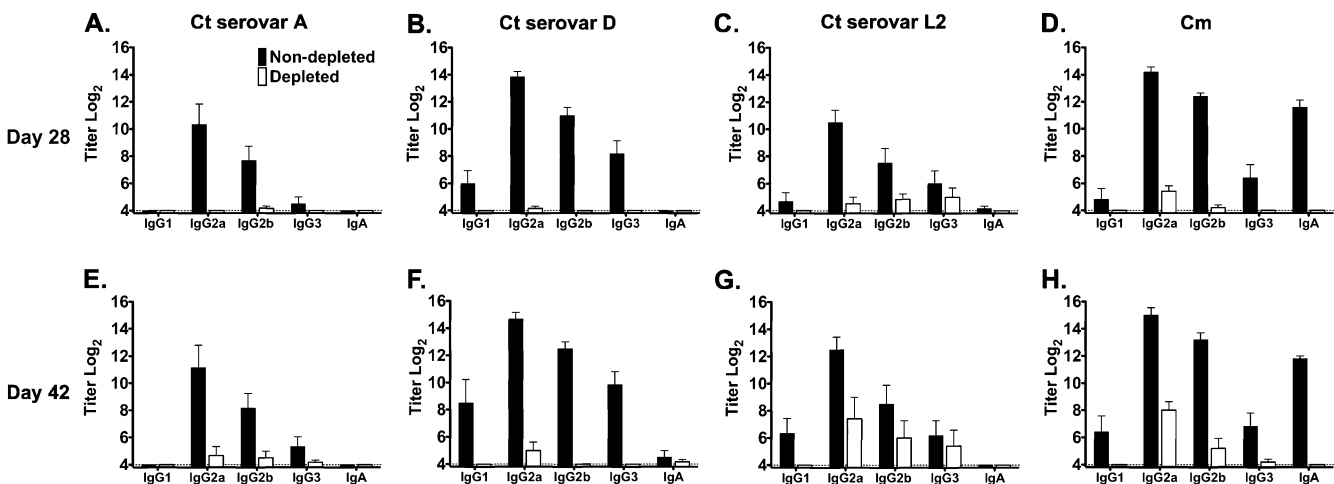

Figure 4. Serologic response of CD4+ T cell–depleted (□) and nondepleted (■) (C3H/HeJ) mice after genital infection with *Chlamydia trachomatis* (Ct) A serovar *(A and E)*, D serovar *(B and F)*, or L2 serovar *(C and G)* or with *C. muridarum* (Cm; *D and H*) (5–6 mice per group). Antibody responses were measured at days 28 *(A–D)* and 42 *(E–H)* of infection. Serum samples were analyzed by enzyme-linked immunosorbent assay for immunoglobulin (Ig) class/subclass antibody responses using the homologous infecting *Chlamydia* strain as antigen. Data are presented as the mean anti-*Chlamydia* titer ± standard error of the mean.
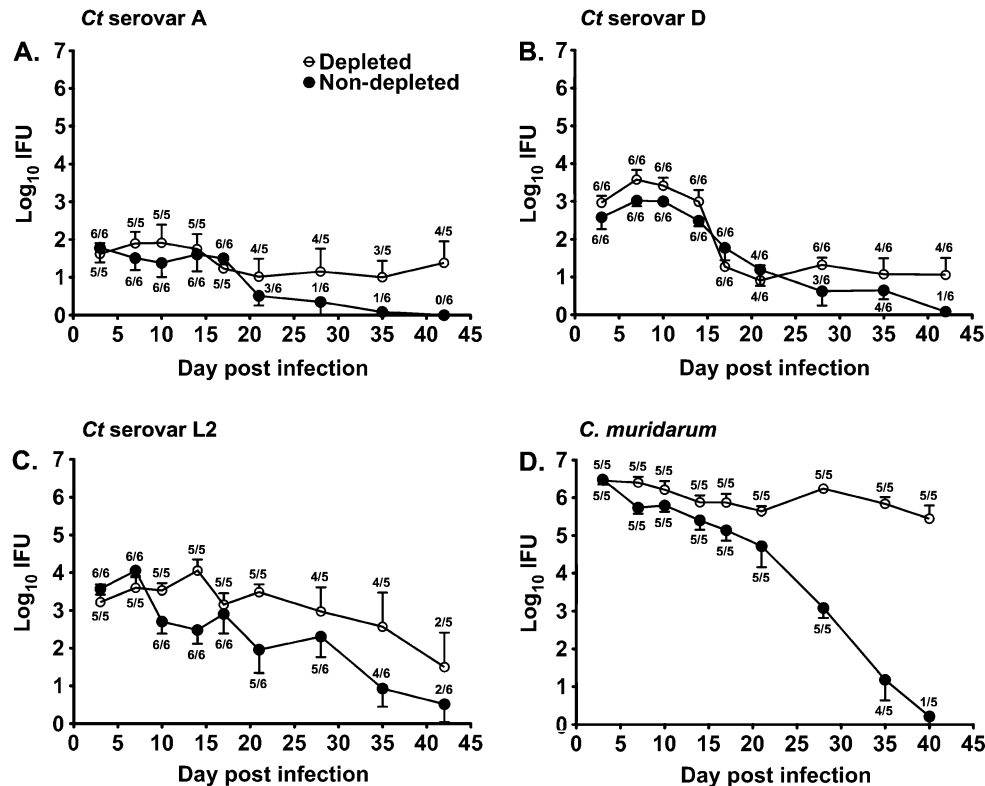
Figure 5. The course of *Chlamydia trachomatis* (Ct) serovar A *(A),* D *(B),* and L2 (C) or *Chlamydia muridarum (D)* genital infection in CD4$^+$ T cell–depleted (⊖) and nondepleted (●) C3H/HeJ mice. Depleted mice were treated with anti-CD4 prior to and throughout the course of infection. Data are presented as mean inclusion forming units (IFUs) ± standard error of the mean for 5–6 mice per group. The number of culture-positive mice/total mice is indicated for each time point.

bacteria during the typical period of infection resolution (days 21–40; $P < .001$).

DISCUSSION

A key observation emerging from numerous mouse model studies is that the characteristics of *C. trachomatis* genital infection are quite different from those of *C. muridarum* infection. For example, compared with *C. muridarum* genital infection *C. trachomatis* infection is characterized by the shedding of far fewer infectious bacteria and by less genital tract inflammation, and the magnitude and duration of infection are strongly influenced by mouse strain. To study adaptive immunity to *C. trachomatis* infection in the mouse we chose a strain of mice (eg, C3H/HeJ), which when challenged with *C. trachomatis*, produces an infection of sufficient magnitude and duration to elicit adaptive immune responses. Using the infection-susceptible C3H/HeJ strain of mice, we made 3 key observations important to understanding immunity to murine *C. trachomatis* infection: (1) unlike the unconditional requirement for CD4$^+$ T cell–mediated responses in resolving *C. muridarum* genital infection, the resolution of murine *C. trachomatis* genital infection was far less dependent on CD4$^+$ T cells; (2) although CD4$^+$ T cells are not necessary to resolve primary infection, adaptive immune responses do develop, and

a marked level of immunity develops following multiple genital infections; and (3) systemic and local anti-chlamydiae IgA responses are mostly absent or delayed.

Our results (Figure 3), as well as those of other investigators, demonstrate that genital infection of mice with *C. trachomatis* elicits significant and substantial cellular and humoral adaptive immune responses [3, 5–7, 9]. Yet unlike *C. muridarum* primary infection, which elicits long-lived protective immunity, the extent of the protective response elicited following primary *C. trachomatis* infection was not significantly different from that of naïve mice (Figure 2C and 2D). Immunity to *C. trachomatis* does, however, increase significantly following the resolution of a second genital infection (Figure 2E and 2F). The lack of correlation between the development of robust adaptive immune responses and inadequate protective immunity is rather curious. Why is it that *C. trachomatis*–infected mice do not exhibit greater immunity following a primary genital infection? The differing characteristics of genital infection may be key to understanding the difference between the striking protective immunity elicited by *C. muridarum* versus *C. trachomatis* infection.

C. muridarum produces a rapidly progressing highly inflammatory infection, whereas murine *C. trachomatis* infection produces nominal inflammation. In *C. muridarum* infection, the marked acute inflammation that is observed in genital tissues early

during infection subsides and is replaced by mononuclear cells as infection resolves [15]. Mononuclear cells localize throughout the genital tissues, but a prominent feature is the clustering of $CD4^+$ T cells [21]. *C. trachomatis* infection is less inflammatory, and it apparently does not produce the $CD4^+$ T cell clusters, which perhaps explains why mice are not protected from reinfection after primary *C. trachomatis* infection.

The difference in the local inflammation elicited by *C. muridarum* and *C. trachomatis* is somewhat surprising given that they share a high degree of genomic synteny [22, 23]. However, one exception is a polymorphic region of the chromosome termed the plasticity zone. Among the genes found in the plasticity zone is the chlamydial cytotoxin, which shares homology with the large clostridial toxins. *C. muridarum* has 3 copies of the intact cytotoxin gene, whereas *C. trachomatis* serovars have multiple mutations in a single copy of the gene, resulting in a truncated cytotoxin [24]. Large clostridial toxins are known inducers of potent inflammatory responses [25–27]; therefore, the marked inflammatory response elicited by *C. muridarum* might result from the effects of the cytotoxin on the genital tissues. The cytotoxin, acting as a mucosal adjuvant, may then help to orchestrate the long-lived protective immunity that follows *C. muridarum* infection. Conversely, the inflammation elicited by the truncated *C. trachomatis* cytotoxin during primary infection may be insufficient to coordinate a potent protective response locally. Although the cytotoxin premise is intriguing, it is also notable that *C. trachomatis* induces a dominant regulatory T cell population that dampens T cell responses [28]. It is not known whether this response is specific for *C. trachomatis* or whether *C. muridarum* causes a similar response. However, an alternative explanation for the dampened protective immunity elicited after *C. trachomatis* infection is that *C. trachomatis* secretes or releases a specific factor(s) that targets these regulatory cells.

In addition to the possible role that the chlamydial cytotoxin may play in modulating the local inflammatory response, the cytotoxin has also been proposed to have a role in limiting the growth of *C. trachomatis* in mouse cells in vitro [29, 30]. In that model, interferon-γ–activated epithelial cells produce p47 GTPases that kill *C. trachomatis*; *C. muridarum*, however, survives in that environment, because the greater abundance of cytotoxin produced by *C. muridarum* inactivates and overcomes the detrimental effects of the p47 GTPases. Whether this protective phenomenon functions in vivo is not known. Furthermore, others have provided intriguing evidence to suggest that additional virulence factors contribute to the pathogenesis of *C. trachomatis* infection in the mouse [19].

One feature of the adaptive immune response that differs between genital *C. muridarum* infection and *C. trachomatis* infection is the delayed production of *Chlamydia*-specific IgA (Figure 3). *C. muridarum* infection elicits vigorous serum and local IgA responses within a few weeks after infection, whereas serum *Chlamydia*-specific IgA is undetectable (or very low in serovar D

infection) and the local IgA response is delayed after *C. trachomatis* primary infection. The difference between the IgA response following *C. trachomatis* and *C. muridarum* infection is remarkable, but whether this difference contributes directly to the delay in the development of protective immunity is not known. Our previous studies using IgA-deficient mice show that IgA is not essential for protective immunity in the *C. muridarum* genital infection model [31], but that does not necessarily imply that it does not function in the *C. trachomatis* model of infection, and perhaps the local antibody response may be key in this model.

There are striking differences in immunity and pathogenesis between the *C. muridarum* and *C. trachomatis* genital infection models, but identifying which model best replicates chlamydial infection, pathogenesis, and immunity of women is not clear. Much like *C. trachomatis* infection of mice, women with genital *C. trachomatis* infection shed low to moderate numbers of infectious bacteria [32], signs of infection (ie, inflammation) are often minimal [33], infection can, but does not always, ascend to cause upper genital tract complication [34, 35], robust cellular and humoral immune responses develop [36], infection resolves in the absence of treatment [37], reinfection is common [38, 39], a level of immunity develops following multiple infections [40], and multiple serovars cause infection. However, there are also caveats that challenge the utility of the murine *C. trachomatis* model. For example, the absence of anti-chlamydial IgA in the *C. trachomatis* mouse infection model is quite different from what is observed in human infection [36, 41]. Furthermore, genital infection of mice with *C. trachomatis* is highly mouse strain dependent, and very substantial challenge doses of chlamydiae are required to produce productive infections [5]. Lastly, because *C. trachomatis* infection in the murine model resolves in the absence of adaptive immunity (Figure 4), it might be more difficult to define protective adaptive immune responses that develop during the natural course of infection. On the other hand, not only does the *C. muridarum* model replicate several aspects of human infection (ie, low infectious dose, ascending infection, resolution of infection without antibiotic treatment, and robust immune responses including IgA), but durable, long-lasting protective immunity develops after infection, facilitating the clear definition of protective mechanisms. Neither model of murine *Chlamydia* infection perfectly replicates human infection, but both have utility in the study of chlamydial pathogenesis and immunity.

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