Chlamydia trachomatis Genital Tract Infection of Antibody-Deficient Gene Knockout Mice

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The importance of antibody-mediated immunity in primary and secondary *Chlamydia trachomatis* genital tract infections was examined by using a definitive model of B-cell deficiency, the μMT/μMT gene knockout mouse. Vaginally infected B-cell-deficient μMT/μMT mice developed a self-limiting primary infection that was indistinguishable from infection of control C57BL/6 mice. Sera and vaginal secretions from infected mice were analyzed for anti-*Chlamydia* antibodies. C57BL/6 mice produced high-titered serum anti-*Chlamydia* immunoglobulin G2a (IgG2a), IgG2b, and IgA antibodies, and vaginal washes contained predominately anti-*Chlamydia* IgA. Serum and vaginal washes from infected B-cell-deficient mice were negative for anti-*Chlamydia* antibody. T-cell proliferation and delayed-type hypersensitivity assays were used as measures of *Chlamydia*-specific cell-mediated immunity and were found to be comparable for C57BL/6 and B-cell-deficient mice. Seventy days following primary infection, mice were rechallenged to assess acquired immunity. B-cell-deficient mice which lack anti-*Chlamydia* antibodies were more susceptible to reinfection than immunocompetent C57BL/6 mice. However, acquired immune resistance was evident in both strains of mice and characterized by decreased shedding of chlamydiae and an infection of shorter duration. Thus, this study demonstrates that cell-mediated immune responses alone were capable of resolving chlamydial infection; however, in the absence of specific antibody, mice were more susceptible to reinfection. Therefore, these data suggest that both humoral and cell-mediated immune responses were important mediators of immune protection in this model, though cell-mediated immune responses appear to play a more dominant role.

Chlamydia trachomatis, an obligate intracellular bacterial pathogen that infects primarily mucosal epithelial cells, is recognized as one of the most common sexually transmitted pathogens. Asymptomatic urogenital infections frequently occur, and if the infections are untreated, the consequences are particularly severe. In women, lower genital tract infection can ascend to cause chronic salpingitis, resulting in ectopic pregnancy or tubal infertility. An effective vaccine would significantly augment efforts to control and prevent sexually transmitted *Chlamydia* infections. However, despite considerable effort, an effective and useful chlamydial vaccine has not been developed.

A current approach for vaccine development focuses on activation of helper T cells and B cells to produce protective antibodies (30, 31, 34, 35). Indeed, there exists considerable indirect evidence suggesting that anti-*Chlamydia* antibodies play an important role in protective immunity. In *C. trachomatis* infections of humans and subhuman primates, protective immunity correlates with the presence of serovar-specific antibodies in local secretions (1, 12, 18, 38, 40). Serovar- and serogroup-specific monoclonal antibodies (MAbs) that react with the major outer membrane protein (MOMP) neutralize chlamydial infectivity for cultured eukaryotic cells, passively protect mice against *Chlamydia* toxicity, and prevent infection of monkey conjunctivae (20, 42). The presence of local (cervical), but not serum, anti-*Chlamydia* immunoglobulin A (IgA) has also been associated with decreased shedding of chlamyd-

iae in women with uncomplicated *C. trachomatis* cervical infection (4). The guinea pig and mouse models of chlamydial genital tract infection also provide evidence for a protective role of antibody (8, 23, 24, 27).

The murine model of chlamydial genital tract infection has proven to be particularly useful because mice can be manipulated to assess directly the role of antibody-mediated immunity and cell-mediated immunity (CMI). Previously, it was shown that T-cell-deficient nude mice and mice lacking major histocompatibility complex class II antigens fail to resolve *Chlamydia* genital tract infection when challenged with the mouse pneumonitis strain (MoPn) of *C. trachomatis* (16, 26). Those studies demonstrate that *Chlamydia*-specific T-cell responses are absolutely required for immunity to chlamydial genital tract infection of the mouse. Supporting this view are the observations that athymic, T-cell-deficient mice resolve *Chlamydia* infection only after reconstitution with lymphocytes or T-cell lines and clones but not with immune sera (10, 11, 19, 21, 37). Furthermore, immune CD4$^+$ T cells transfer significant anti-*Chlamydia* immunity to immunocompetent naive mice (29). Therefore, cell-mediated immune responses comprise the necessary elements needed to resolve chlamydial genital tract infection.

The role of antibody in immune protection in the murine model is more controversial. Mice depleted of B cells by chronic anti-μ antibody treatment resolve infection and are protected from reinfection (22), suggesting that *Chlamydia*-specific antibody is not an essential component of the protective immune response. However, because neonatal anti-μ suppression of B cells is incomplete, a possible contribution of remaining B cells to protection cannot be ruled out (15). Furthermore, the reconstitution of T-cell-deficient recipients (athymic mice) by adoptive transfer of T cells may activate

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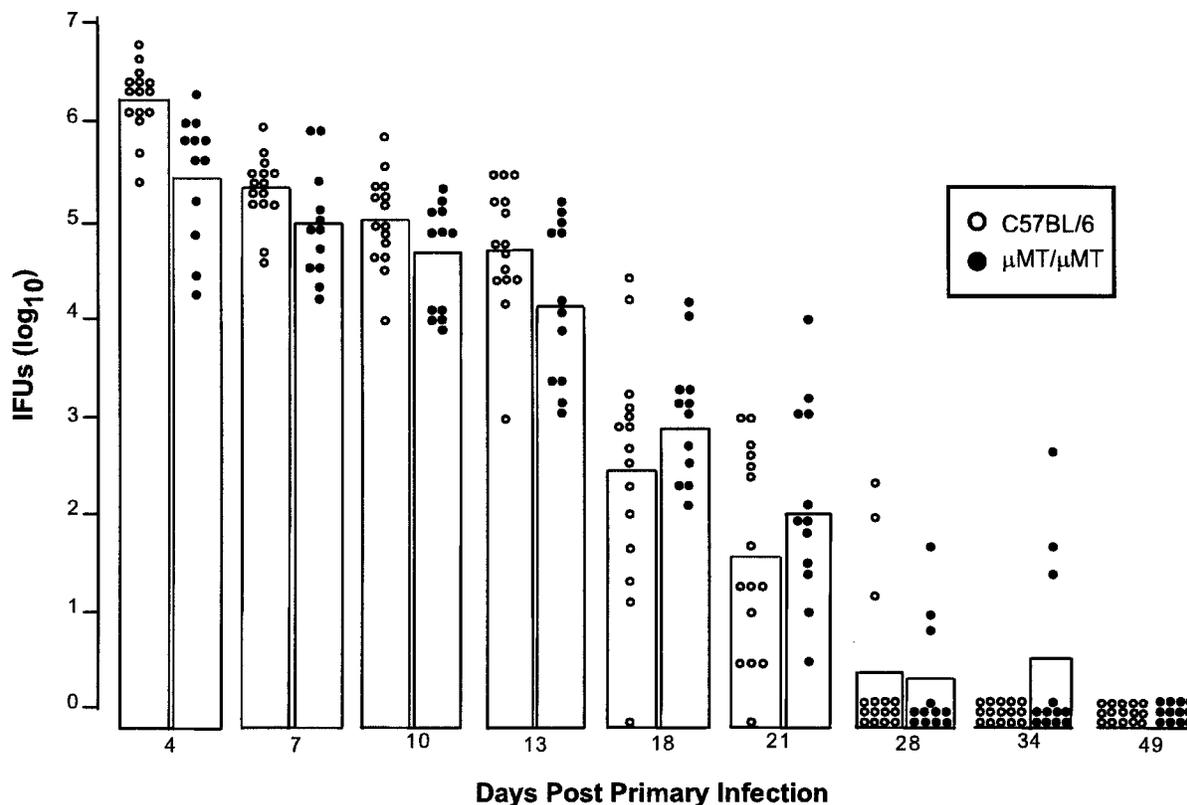
FIG. 1. Time course of primary *Chlamydia* genital tract infection in C57BL/6 and B-cell-deficient μMT/μMT mice. Infection was monitored by swabbing the vaginal vault at various times following infection with 100 ID$_{50}$ of *C. trachomatis* MoPn and enumerating IFU by isolation onto HeLa cell monolayers. Inclusions were visualized by indirect immunofluorescence with a genus-specific antilipopolysaccharide MAb (EIV-H1) and FITC-tagged goat anti-mouse IgG. Data are presented as IFU for individual mice, and bars indicate the mean IFU for each group of mice.

endogenous B cells to produce protective antibodies, thus confounding the interpretation of the results (13). Also, in a recent study, *Chlamydia*-specific antibody was shown to affect the course of chlamydial upper genital tract infection (8).

Defining the significance of antibody-mediated immunity and CMI in resolving *Chlamydia* infection is clearly an issue with obvious implications for vaccine design. Therefore, in this study, we reexamined the role of antibody-mediated immunity in experimental *Chlamydia* genital tract infection by using a definitive model of B-cell deficiency, the μMT/μMT mouse. We found that cell-mediated immune responses alone were sufficient to resolve primary and secondary chlamydial infections. However, animals that lacked antibody were more susceptible to reinfection, suggesting a role for antibody in preventing mucosal colonization by *Chlamydia*.

MATERIALS AND METHODS

Growth, purification, and enumeration of chlamydiae. The mouse pneumonitis strain of *C. trachomatis* (MoPn) was grown in HeLa 229 cells, and elementary bodies (EBs) were purified by use of discontinuous gradients of diatrizoate meglumine (Renografin 76; Squibb Diagnostics, New Brunswick, N.J.) as described previously (7). Infectivity of purified EBs was assayed by enumeration of chlamydial inclusion-forming units (IFU) on monolayers of HeLa 229 cells grown in 96-well tissue culture plates. The same seed stock of MoPn was used throughout the study.

Animals. Female C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, Maine). B-cell-deficient μMT/μMT mice (14) were obtained from R. Schwartz, National Institute of Allergy and Infectious Diseases, National Institutes of Health, and were bred and maintained at the Rocky Mountain Laboratory animal facilities. B-cell maturation is arrested in μMT/μMT mice at the pre-B-cell stage, and thus these mice lack surface IgD and IgM and fail to

produce detectable levels of immunoglobulin (14). Mice 8 to 14 weeks of age were used throughout the study.

Primary genital tract infection. Mice received 2.5 mg of Depo-provera (medroxyprogesterone acetate) subcutaneously at 10 and 3 days prior to vaginal infection. Mice were infected by placing 5 μl of 250 mM sucrose–10 mM sodium phosphate–5 mM L-glutamic acid (pH 7.2) (SPG) containing 1,500 IFU (100 50% infectious doses [ID$_{50}$]) of MoPn into the vaginal vault. The course of infection was monitored by swabbing the vaginal vault with a Calgiswab (Spectrum Medical Industries, Los Angeles, Calif.) at various times postinfection and enumerating IFU by isolation onto HeLa cell monolayers. Inclusions were visualized by indirect immunofluorescence using the genus-specific antilipopolysaccharide MAb EVI-H1 and fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgG.

Secondary infectious challenge. Infection of immunocompetent mice results in a self-limiting infection that resolves in approximately 4 weeks (16). To determine the immune status of C57BL/6 and μMT/μMT mice following primary infection, groups of mice that had recovered from primary infection were challenged with 100 ID$_{50}$ of MoPn 70 days post-primary infection. The mice were treated with progesterone 10 and 3 days prior to challenge, and infection was monitored by assessing IFU recovered from vaginal vault swabs as described above.

Flow cytometry. Spleen cells from naive and infected C57BL/6 and μMT/μMT mice were stained with FITC-conjugated anti-B-cell (anti-B220, clone RA36B2), anti-T-cell (anti-Thy-1.2, clone 53-2.1), anti-CD8 (anti-Lyt-2, clone 169.4), or antimacrophage (anti-MAC-1 α unit, clone M1/70.15.11.5.HL) antibodies or phycoerythrin-conjugated anti-CD4 (anti-L3T4, clone GK1.5) or anti-NK cell (anti-NK-1.1, clone PK136) antibodies and analyzed with a FACStar instrument (Becton Dickinson Immunocytometry Systems, San Jose, Calif.). An FITC-labeled mouse anti-rat kappa light-chain antibody (clone MAR 18.5) was used as a negative control. Each sample was collected in a list mode file of 10,000 events, and data are presented as the percentages of each cell population in the spleens of naive or infected C57BL/6 and μMT/μMT mice.

Analysis of serum and secretory antibody responses. Serum and secretory class and subclass anti-*Chlamydia* antibody responses were assayed by enzyme-linked immunosorbent assay (ELISA) as described previously (16). Sera and secretions collected from mice prior to infection were used as negative controls.

Fourfold serial dilutions of sera were prepared, and *Chlamydia*-specific antibody titers were expressed as the highest serum dilutions giving an absorbance (optical density at 405 nm) of at least 0.3 and 3 times that of preimmune sera (optical density consistently <0.1). Vaginal secretions were collected by rinsing the vaginal vault twice with 60 μl of 10 mM phosphate-buffered saline containing 0.5% bovine serum albumin. Vaginal washes were always collected at least 1 day prior to the swabbing of the vaginal vault for the enumeration of IFU. We found that washes collected in this manner were generally free of serum-derived IgG antibodies and contained primarily IgA. Washes from each animal were pooled, clarified by centrifugation, and diluted further with ELISA buffer (0.05 M Tris buffer [pH 7.5] with 0.15 M NaCl) to bring the total volume to 800 μl. Fourfold serial dilutions were prepared and analyzed by ELISA as described above. Because the volume of vaginal fluid cannot be determined accurately, the data are presented as the number of animals with positive responses divided by the total number tested.

Preparation of APCs. Antigen-presenting cells (APCs) were prepared as described previously (33). Spleens were harvested from naive C57BL/6 and μMT/μMT mice, single-cell suspensions were prepared, and erythrocytes were lysed with 0.17 M Tris-buffered ammonium chloride. Splenocytes (10^7/ml) in Dulbecco's modified Eagle medium (DMEM) were incubated with heat-killed chlamydiae at a multiplicity of infection of 10 IFU per cell in a 37°C shaking water bath for 1 h. APCs were irradiated (3,000 rad) in a ^{137}Cs γ irradiator, washed twice with phosphate-buffered balanced salt solution (pH 7.2) containing 5% fetal calf serum (FCS), and resuspended in DMEM containing 10% FCS (DMEM-10) to a concentration of 10^7 cells/ml. APCs were plated at 100 μl per well in 96-well flat-bottom plates for T-cell proliferation assays.

T-cell proliferation assay. At 0 and 33 days, naive and infected mice were sacrificed, their spleens were harvested, and single-cell suspensions were prepared. Erythrocytes were lysed with 0.17 M Tris-buffered ammonium chloride. Splenic leukocytes were resuspended in 10 mM phosphate-buffered saline (pH 7.2) containing 5% FCS, and T cells were isolated by using an IsoCell mouse T-cell isolation kit (Pierce, Rockford, Ill.) according to the manufacturer's protocol. Splenic T cells from five naive (day 0) or five immune (day 33) mice were pooled, suspended in DMEM-10, and added to 96-well plates (2.5×10^5 cells/well) containing APCs (see above). Five days following incubation at 37°C in 5% CO_2, 20 μl of DMEM-10 containing 1 μCi of [^{3}H]thymidine (New England Nuclear, Du Pont, Wilmington, Del.) was added to each well, and the plates were incubated for an additional 18 h. The cells were harvested, and radioactive incorporation was measured with a liquid scintillation counter (LS6000LL; Beckman Instruments, Palo Alto, Calif.). Data are expressed as the mean Δcpm ± standard deviations (SD) of triplicate cultures, where Δcpm is the difference in counts per minute of antigen-stimulated and -nonstimulated cultures.

Cytokine assay. Interleukin 6 (IL-6) and gamma interferon (IFN-γ) were measured by ELISA as described previously (29). For these experiments, splenic $CD4^+$ T cells were isolated from C57BL/6 and B-cell-deficient mice at 33 days following primary infection. T cells were cultured with antigen-pulsed APCs (described above), and 72-h culture supernatants were harvested and assayed for IL-6 and IFN-γ.

DTH responses. Delayed-type hypersensitivity (DTH) responses were assessed by injecting hind footpads of naive and infected C57BL/6 and μMT/μMT mice with 25 μl of either SPG or heat-inactivated (80°C, 30 min) MoPn EBs (0.8 μg/ml of SPG), as described previously (16). Groups of mice were tested before infection (day 0) and at 41 days postinfection. Footpad swelling represents the difference in footpad thickness measured before inoculation and at 24 h postinoculation. Data are presented as the mean footpad swelling of at least five mice per time point ± SD. The histopathology of the anti-*Chlamydia* DTH response has been described in detail previously (16).

Statistical analysis. Student's *t* test of log-transformed data was used to analyze differences between mean IFU counts and differences between T-cell proliferation responses of control and experimental groups. Fisher's exact test was used to analyze differences in susceptibility to reinfection between groups of C57BL/6 mice and B-cell-deficient mice which had recovered from a primary chlamydial infection.

RESULTS

Course of *Chlamydia* genital tract infection in control and B-cell-deficient mice. Vaginally infected B-cell-deficient mice and immunocompetent C57BL/6 mice developed self-limiting infections that resolved by about 4 weeks after infectious challenge (Fig. 1). The shedding of infectious chlamydiae from C57BL/6 or B-cell-deficient mice was not significantly different at any time point postinfection ($P > 0.05$). By 34 days postinfection, 15 of 15 C57BL/6 mice and 9 of 12 B-cell-deficient mice were culture negative, and by 42 days postinfection, all mice had resolved the infection.

Histopathological analyses of hematoxylin-eosin-stained genital tract tissues from the two strains of infected mice were

TABLE 1. Fluorescence-activated cell sorter analysis of splenocytes from *Chlamydia*-infected C57BL/6 and μMT/μMT mice

Cell type	Splenocyte composition on the indicated day postinfection[a]			
	0		41	
	C57BL/6	μMT/μMT	C57BL/6	μMT/μMT
B cells	54.8	≤0.8	62.3	≤1
T cells	38.5	49.4	30.3	31.3
$CD4^+$ T cells	21.3	28.6	18.8	15.5
$CD8^+$ T cells	15.9	19.3	10.1	13.7
NK cells	2.5	5.5	3.1	3.4
Macrophages	3.1	13.8	4.2	28.0

[a] Percentage of each cell type present in the spleens of control (C57BL/6) and B-cell-deficient μMT/μMT mice.

similar and not different from that reported in previous studies (16). A marked subacute inflammatory response of the submucosal epithelium of the vagina, cervix, uterine horns, and oviducts developed during the course of infection and resolved by 42 days postinfection. However, oviduct ectasia and fibrosis which resulted in hydrosalpinx developed in both strains of mice (data not shown).

Flow cytometry of splenocytes from control and B-cell-deficient mice. To verify that μMT/μMT mice were B cell deficient, flow cytometric analysis was performed on splenic leukocytes from μMT/μMT mice before infection and after resolution of genital tract infection (Table 1). B cells were not detected in μMT/μMT mice before or after *Chlamydia* infection, whereas a large percentage of splenic leukocytes from control C57BL/6 mice were B cells. Furthermore, the percentages of T cells, $CD4^+$ T cells, $CD8^+$ T cells, and NK cells were similar in the two strains of mice. A difference in the number of macrophages in the splenic leukocyte populations of control and μMT/μMT mice was noted. The influence of this increased number of macrophages on the ability of μMT/μMT mice to resolve *Chlamydia* infection is not known, but the increase may be a compensatory response for the lack of antigen-presenting B cells in these mice.

***Chlamydia*-specific serum and mucosal antibody responses in control and B-cell-deficient mice.** To determine if B-cell-deficient mice were incapable of producing both systemic (serum) and local (vaginal) anti-*Chlamydia* antibodies, sera and vaginal washes were analyzed by ELISA using MoPn EBs as an antigen and monospecific class- and subclass-specific anti-mouse immunoglobulin sera. Infected C57BL/6 mice produced primarily IgG2a, IgG2b, and IgA serum anti-*Chlamydia* antibodies (Table 2). Serum titers of IgM and IgG3 were low, and IgG1 anti-*Chlamydia* antibodies were rarely produced. In contrast, serum anti-*Chlamydia* antibodies were not detected in *Chlamydia*-infected B-cell-deficient mice.

Because *Chlamydia* infection localizes to the genital tract epithelium, it is important to define the local (mucosal) antibody response (Table 3). *Chlamydia*-specific IgA antibodies were consistently detected in vaginal washes of infected C57BL/6 mice. Vaginal washes were rarely positive for IgG2a and IgG2b anti-*Chlamydia* antibodies, and IgM, IgG1, and IgG3 anti-*Chlamydia* antibodies were never detected. Vaginal washes from B-cell-deficient mice were consistently negative for anti-*Chlamydia* antibodies, even when the animals had resolved the infection (day 40 postinfection). Collectively, these data demonstrate that mutant mice in which B-cell development is arrested at the stage of pre-B-cell maturation (14) produced neither serum nor mucosal anti-*Chlamydia* antibod-

TABLE 2. Anti-*Chlamydia* serologic response[a]

Mouse strain	Days postinfection	Anti-*Chlamydia* titer (log$_2$)[b]					
		IgM	IgG1	IgG2a	IgG2b	IgG3	IgA
C57BL/6	7	4.7 (0.7)	<3	<3	4.7 (1.0)	<3	<3
	28	3.9 (0.9)	<3	12.2 (0.9)	13.2 (0.8)	6.9 (2.8)	9.9 (1.6)
	40	3.8 (0.9)	<3	12.3 (0.7)	13.7 (1.2)	6.4 (3.0)	12.4 (0.8)
	54	3.4 (1.0)	3.7 (2.2)	12.7 (0.9)	13.6 (1.1)	5.9 (3.1)	12.3 (0.8)
μMT/μMT	7	<3	<3	<3	<3	<3	<3
	28	<3	<3	<3	<3	<3	<3
	40	<3	<3	<3	<3	<3	<3
	54	<3	<3	<3	<3	<3	<3

[a] Sera were collected at various times postinfection and analyzed by ELISA for anti-*Chlamydia* antibodies. Sera collected before infection were uniformly negative (<3.0 log$_2$). The initial dilution of sera was 1/8; therefore, if the initial dilution was not positive, a value of <3 log$_2$ was assigned for that sample.
[b] Mean ($\pm$ SD) of 10 mice/group.

ies, but they resolved genital tract infection similarly to immunocompetent mice.

Cell-mediated immune responses following genital tract infection of control and B-cell-deficient mice. Lymphocyte proliferation and DTH assays were used as in vitro and in vivo measures of CMI, respectively. Splenic T cells from C57BL/6 and B-cell-deficient μMT/μMT mice that had resolved a primary chlamydial genital tract infection produced comparable levels of T-cell proliferation and T helper cell type 1 (Th1) and Th2 cytokines (IFN-γ and IL-6, respectively) when stimulated with autologous APCs and heat-inactivated MoPn EBs (Fig. 2A and B). IL-4 and IL-5 were not detected, which is characteristic of this model (29). Also, positive DTH responses to heat-inactivated EBs in the two strains of mice were comparable (Fig. 2C). Therefore, cell-mediated immune responses developed in B-cell-deficient mice following chlamydial genital tract infection, and those responses were similar to that of immunocompetent C57BL/6 mice.

Acquired immunity to chlamydial genital tract infection. It was clear from our earlier experiments (Fig. 1) that *Chlamydia*-specific antibody was not necessary to bring about the resolution of primary chlamydial genital tract infection, but those studies did not address whether antibody was involved in acquired resistance to reinfection. To determine if the lack of *Chlamydia*-specific antibody influenced the ability of mice to resist a secondary infectious challenge, groups of C57BL/6 and B-cell-deficient μMT/μMT mice that had recovered from a primary chlamydial genital tract infection were rechallenged vaginally with 100 ID$_{50}$ of *Chlamydia*. Both C57BL/6 and B-cell-deficient μMT/μMT mice developed an acquired immune resistance following primary infection, which was characterized by decreased shedding of infectious chlamydiae and an infection of shorter duration. However, mice with vaginal anti-*Chlamydia* antibody (C57BL/6) were more resistant to colonization (33% infected) ($P = 0.005$ on day 5 and $P = 0.008$ on day 8 post-secondary challenge) than B-cell-deficient μMT/μMT mice (100% infected) and shed fewer chlamydiae ($P < 0.05$ on day 5 post-secondary challenge) (Fig. 3). Those results indicate that cell-mediated immune responses played a dominant role in immune protection but also suggest that antibody might contribute.

DISCUSSION

Currently, there is interest in pursuing vaccination as a means of controlling the spread of the sexually transmitted bacterial pathogen *C. trachomatis* and the development of the serious sequelae that often follow urogenital chlamydial infec-

tion. The paucity of success of previous vaccination efforts has not been due to a lack of effort (3, 9, 17, 31, 35, 38, 39) but instead may be the result of our incomplete understanding of what comprises protective immunity to chlamydial urogenital infection. One approach to resolving this issue is to use an animal model that mimics human infection. Vaginal inoculation of mice with *C. trachomatis* produces a genital infection similar to urogenital chlamydial infection of women and is a model in which acquired immunity can be studied.

Our recent studies, and those of others, have demonstrated the importance of major histocompatibility complex class II-restricted T-cell responses in resolving primary chlamydial genital tract infection (11, 16, 21, 29) but have not adequately addressed those immune responses that provide resistance to reinfection. Similarly, the delineation of the relative importance of Th1- and Th2-type T helper cell responses has not been conclusive, although Th1 T-cell clones have been shown to resolve infection in T-cell-deficient athymic mice (11) and Th1-type T helper cell responses correlate with the resolution of infection (6, 41). In principle, because of the obligate intracellular lifestyle of *Chlamydia*, it is unlikely that *Chlamydia*-specific antibodies play a major role in resolving primary infection of a naive animal. Conversely, though, the presence of specific antibodies in genital tract secretions at the time of infectious challenge might contribute significantly to acquired immunity by blocking colonization and subsequent infection. Therefore, the murine model of chlamydial genital tract infection is a useful tool for studying not only immune responses

TABLE 3. Anti-*Chlamydia* antibody response in genital tract secretions[a]

Mouse strain	Days postinfection	Animals positive by ELISA (no. positive/total no. tested)		
		IgG2a	IgG2b	IgA
C57BL/6	7	0/10	0/10	0/10
	32	0/10	1/10	10/10
	54	1/10	1/10	10/10
μMT/μMT	7	0/10	0/10	0/10
	32	0/10	0/10	0/10
	54	0/10	0/10	0/10

[a] Vaginal washes were collected and analyzed as described in Materials and Methods. Data are shown for IgG2a, IgG2b, and IgA only, because anti-*Chlamydia* IgM, IgG1, and IgG3 were never detected in vaginal washes. Vaginal washes obtained prior to infection were negative for anti-*Chlamydia* antibodies.
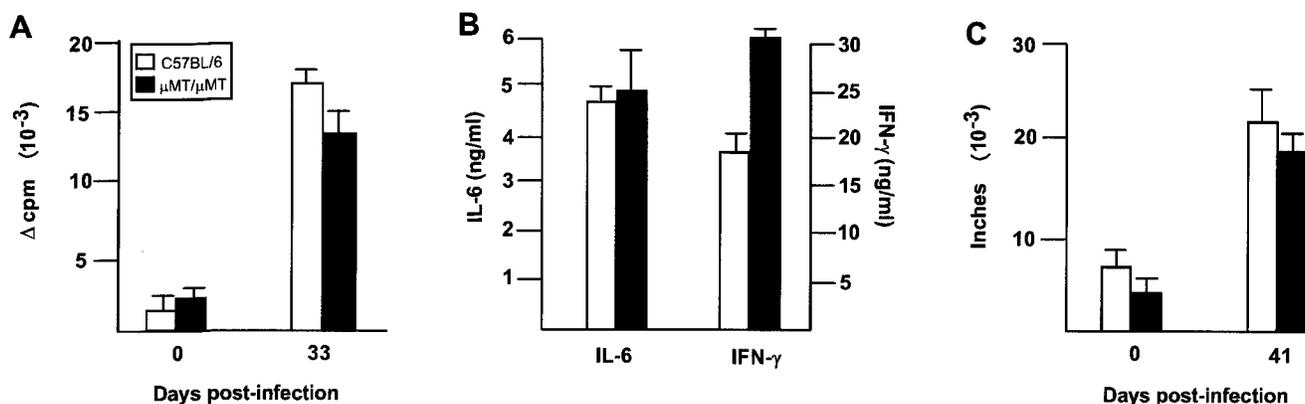
FIG. 2. Development of cell-mediated immune responses in C57BL/6 and B-cell-deficient μMT/μMT mice following primary *Chlamydia* infection. (A) T-cell proliferative response at 33 days following primary *Chlamydia* infection. Data are presented as the mean Δcpm ± SD of triplicate cultures. (B) IL-6 and IFN-γ secretion by splenic CD4$^+$ T cells. Cytokine levels were determined from 72-h culture supernatants. Data are presented as mean values for triplicate samples ± SD. (C) DTH response of mice at 41 days following primary infection. Footpad swelling was measured at 24 h following antigen inoculation. Data are presented for antigen inoculation only, because swelling was not observed following buffer inoculation. Data are presented as the means ± SD for five mice per group.

that contribute to the resolution of infection, but also those that function in acquired resistance to reinfection.

In this study, we used a definitive model of antibody deficiency, the μMT/μMT mouse, to determine if antibody was necessary to resolve primary chlamydial infection or to protect from reinfection. Our results corroborated and extended previous findings showing that mice rendered B cell deficient by anti-μ antibody treatment resolve infection similarly to control mice (22) (Fig. 1). However, unlike the findings of previous studies, we found that antibody-positive mice were more resistant to infection upon secondary challenge than animals which lacked *Chlamydia*-specific antibody, suggesting that antibody might be involved in acquired immune resistance. Note, however, that even though antibody-deficient mice were more readily colonized than controls, acquired protective immunity was evident and was characterized by decreased shedding of infectious chlamydiae and an infection of shorter duration.

The cellular aspects of the immune response that resolves infection in B-cell-deficient mice are not understood but could involve either cell-mediated immune responses that typically function in immunocompetent hosts or compensatory immune responses that are activated following infection of these immunodeficient animals. The importance of T cells in resolving primary infection has been demonstrated previously (10, 11, 21, 26, 29). Those studies showed that both CD4$^+$ and CD8$^+$ T cells confer a measure of protective immunity but that in all instances *Chlamydia*-reactive CD4$^+$ T cells are more efficient in resolving infection. Recently, we demonstrated the importance of CD4$^+$ T cells, but not CD8$^+$ T cells, in acquired immunity to chlamydial genital tract infection by adoptively immunizing mice with purified T-cell subpopulations (29). That study confirms the important role of CD4$^+$ T cells in protective immunity and demonstrates that T-cell immunity is quite long-lived. The mechanism(s) by which CD4$^+$ T cells confer immune protection is not known, but secretion of the *Chlamydia*-inhibitory cytokine IFN-γ is one possibility. The in vitro and in vivo inhibitory effects of IFN-γ have been documented previously. For example, IFN-γ inhibits the growth of *Chlamydia* in vitro when added before or at the time of infection (2) and promotes the resolution of genital tract infection in T-cell-deficient (nude) mice when passively administered in vivo (25). Although we do not understand the precise protec-

tive immune mechanisms that function to resolve *Chlamydia* infection, clearly, aspects of CMI are involved.

The conclusion that CMI alone (without antibody) resolves *Chlamydia* infection does not necessarily contradict the observation that antibody plays a protective role. Our results show that following secondary infectious challenge, antibody-negative mice are more readily colonized by chlamydiae than are antibody-positive mice (Fig. 3). Those results provide indirect evidence that antibody is protective. The mechanism(s) by which antibodies protect in vivo is not understood, but in vitro, protective antibodies function by blocking attachment of chlamydiae to susceptible host cells (32, 36). If a similar mechanism occurs in vivo, then colonization and subsequent infection could be prevented by local antibodies. Although our results support that premise, they also reveal that sterilizing immunity through antibody neutralization will be very difficult to achieve. For example, even under conditions optimal for induction of
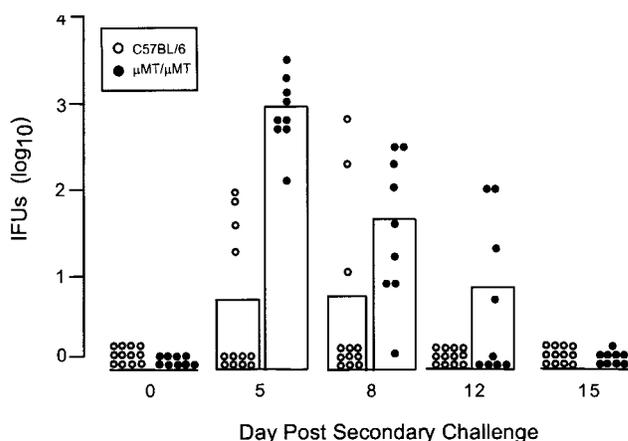

FIG. 3. Acquired immunity of C57BL/6 and B-cell-deficient μMT/μMT mice to chlamydial genital tract infection. Mice were infected with 100 ID$_{50}$ of MoPn EBs and allowed to resolve their primary infection. Seventy days following primary infection, groups of mice were rechallenged vaginally with 100 ID$_{50}$ of MoPn. Infection was monitored as described in Materials and Methods. The mean numbers of recoverable IFU for each animal (circles) and for each group of animals (bars) are shown for the culture times postinfection.

protective immunity (i.e., recovery from a primary infection), where the immune response is polyclonal in regard to bacterial antigens, antibodies, and cell populations, protection from colonization is not absolute (67% protected) (Fig. 3). Although we do not understand why some antibody-positive mice become reinfected, it might be explained by the composition of the inoculum. The purification of *Chlamydia* EBs results in preparations composed of infectious and noninfectious bacteria, and the ratio of noninfectious to infectious EBs has been estimated to approach 1,000:1 (5). If noninfectious chlamydiae bind neutralizing antibody, then the local antibody response might be overwhelmed by the challenge inoculum, resulting in ineffective neutralization of the infectious organism. Alternatively, complete in vivo neutralization, like in vitro neutralization, might be very difficult to achieve and might require quite high concentrations of specific antibody (5).

The susceptibility of B-cell-deficient mice to secondary infectious challenge might also be explained by altered CMI instead of a lack of antibody. For example, a delay in the cell-mediated immune response, a response dominated by different cell populations, or a lack of antigen-presenting B cells might explain the decreased resistance to reinfection. Our data show that prior to secondary challenge, measures of CMI (T-cell proliferation, cytokine production, and DTH responses) in C57BL/6 and B-cell-deficient mice were similar (Fig. 2). However, those assays neither detect subtle differences in CMI nor address the kinetics of the secondary cell-mediated immune response. Therefore, although susceptibility to reinfection in B-cell-deficient mice correlated with the lack of antibody, we cannot conclusively eliminate altered cell-mediated immune responses as a possible cause. The mechanism(s) by which CMI protects against reinfection is not known but might involve the inhibition of intracellular chlamydial growth by cytokines such as IFN-γ (28) rather than the blocking of attachment and subsequent entry of chlamydiae into host cells.

Our results raise questions regarding the therapeutic value of a vaccine targeted solely at eliciting neutralizing antibody. Although a vaccine that produces complete immunity by preventing colonization would be ideal, it is unlikely that effective vaccination against *Chlamydia* genital tract infection will be achieved with a vaccine designed to elicit only local (mucosal) neutralizing antibodies. Possibly, a better vaccine strategy might be to focus on elements of *Chlamydia* that elicit both protective CMI and neutralizing antibodies. Antibodies to the chlamydial MOMP have previously been shown to neutralize chlamydial infectivity (20, 42), but the specificities of the antibodies that confer protection in this infection model have not been identified. Furthermore, the effector cells of CMI that resolve infection have not been thoroughly characterized, nor have their antigenic specificities been determined. The chlamydial MOMP is a likely antigen to which protective immunity is elicited, but further studies are needed to determine if MOMP can stimulate both protective antibody and CMI and whether those responses are directed to unique or similar antigenic determinants. Lastly, once protective antigens are identified, it must be determined if they can be administered in a manner that confers protective immunity to a naive host. Mice provide a useful animal model in which these questions can be experimentally addressed, and further characterization of infection and immunity in this model may provide information that is applicable to the pathogenesis of human chlamydial infection.

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