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Gene Knockout B Cell-Deficient Mice Demonstrate That B Cells Play an Important Role in the Initiation of T Cell Responses to Chlamydia trachomatis (Mouse Pneumonitis) Lung Infection

Xi Yang2 and Robert C. Brunham

T cell-mediated immunity as measured by delayed-type hypersensitivity, and IFN-γ production has been shown to be critical for host defense against Chlamydia trachomatis infection in both human and animal studies. Using gene-targeted B cell-deficient mice, we examined the role of B cells in protective immunity to C. trachomatis (mouse pneumonitis) (MoPn) lung infection. B cell-deficient mice were observed to have a significantly higher mortality rate and in vivo chlamydial growth than did wild-type mice following MoPn lung infection. Interestingly, B cell-deficient mice not only lacked Ab responses but also failed to mount an efficient delayed-type hypersensitivity response following chlamydial lung infection. In contrast to results obtained from MoPn-infected wild-type C57BL/6 mice, spleen cells from infected B cell-deficient mice failed to produce Th1-related (IFN-γ) or Th2-related (IL-6 and IL-10) cytokines after Chlamydia-specific in vitro restimulation. Moreover, unlike wild-type mice, B cell-deficient mice were not immune to rechallenge infection following recovery from primary chlamydial infection. The data indicate that B cells play an important role in host defense to primary and secondary chlamydial infection and suggest that B cells are crucial for the initiation of early T cell responses to chlamydial infection. This study provides evidence for the role of B cells in the in vivo priming of T cells during infection with the intracellular bacterial pathogen, C. trachomatis. The Journal of Immunology, 1998, 161: 1439–1446.

Several recent studies have highlighted the role of T cells in protective immunity to Chlamydia trachomatis infection. Both epidemiologic studies in humans and animal models of infection have demonstrated the extreme importance of CD4+ T cell responses in host defense against chlamydial infection (reviewed in Refs. 1 and 2). For example, athymic nude mice showed a more severe and chronic course of respiratory and genital chlamydial infection than normal control mice (3). Furthermore, depletion of CD4+ T cells significantly impaired clearance of genital and pulmonary chlamydial infection in mice (4) and adoptive transfer of Chlamydia-specific CD4+ T cell lines or clones or freshly isolated polyclonal CD4+ T cells protected both immunocompromised and normal mice from genital and respiratory infection (5–7). In particular, adoptive transfer of thymocyte or Th1 clones provided significant protection in SCID mice (7). Epidemiologic analysis of affected humans that was conducted in the Gambia showed that scarring trachoma, in contrast to healing trachoma, was correlated with impairment in cell-mediated immunity (T cell-proliferative responses and Ag-specific IFN-γ production) (8–10). Moreover, using a murine model of chlamydial lung infection, we previously reported that strong cell-mediated immune response (delayed-type hypersensitivity (DTH) and IFN-γ production were associated with more rapid clearance of the mouse pneumonitis (MoPn) from the lung (11). Using the same model, we also demonstrated that αβ TCR, but not γδ TCR, T cells play the dominant role in host defense against chlamydial infection (12). Although chlamydia elicits strong local and systemic Ab responses in both humans and animals, the role that such Abs play in protective immunity to chlamydial infection is uncertain (13–15). Notably, a series of studies conducted over a decade ago showed that B cell-deficient mice generated by chronic treatment with anti-μ Abs were able to resolve primary and secondary MoPn infection as readily as normal mice following genital and respiratory challenge infections, suggesting that B cells are not essential for host defense against chlamydial infection (16, 17).

Since chronic anti-μ Ab treatment involves in vivo delivery of an extraordinary amount of Ab, it may alter the microenvironment and concomitant cell-to-cell interactions that occur during chlamydial infection. As well, in spite of the large amount of Ab used, anti-μ Ab treatment may not completely deplete B cell populations in vivo, and although serum IgM is reduced to undetectable levels, IgG is generally readily detectable, albeit at levels 10- to 1000-fold lower than normal (18). Therefore, additional studies using alternative approaches to deplete B lymphocytes are needed to elucidate whether B cells play a role in host defense against chlamydial infection. Accordingly, we used B cell-deficient mice (μMT) generated by disruption of the transmembrane portion of the μ-chain gene (19) to reexamine the role played by B cells in the resolution of primary and resistance to secondary MoPn lung infection. In striking contrast to previous reports regarding chlamydial infection in anti-μ Ab-treated mice, gene knockout B cell-deficient (μMT)

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3 Abbreviations used in this paper: DTH, delayed-type hypersensitivity; MoPn, mouse pneumonitis; EBs, elementary bodies; IFUs, inclusion-forming units.

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mice showed dramatically increased mortality and in vivo chlamydial growth following MoPn lung infection. B cell-deficient (μMT) mice showed complete absence of Ab responses and greatly impaired cell-mediated immune responses (DTH) to chlamydial elementary bodies (EBs). Most strikingly, spleen cells obtained from MoPn-infected μMT mice failed to produce T cell-derived cytokines after in vitro EB restimulation. The results argue that B cells play an essential role in host defense to chlamydial lung infection and suggest that B cells are crucial for the initiation of T cell responses during chlamydial infection. The study provides further evidence that B cells are necessary for T cell priming in vivo following infection with the intracellular pathogen, C. trachomatis.

Materials and Methods

Mice

Female B cell-deficient (μMT) mice with a C57BL/6 genetic background (C57BL/6-1gh-6tm1Cgn) were purchased from The Jackson Laboratory (Bar Harbor, ME). Age- and sex-matched wild-type C57BL/6 mice were purchased from The Jackson Laboratory or Charles River Canada (St. Constant, Quebec, Canada). All the animals were maintained under specific pathogen-free conditions in the central animal facilities of the University of Manitoba. Mice 8 to 14 wk of age were used for the study.

Mouse pneumonitis

The mouse pneumonitis biovar (Nigg strain) of C. trachomatis (MoPn) was grown in HeLa 229 cells and purified by discontinuous density gradient centrifugation. Infectivity of the purified EBs was titrated by infection of HeLa cell monolayers followed by methanol fixation of cells and enumeration of inclusions which were stained by a genus-specific mAb and horseradish peroxidase conjugate. The procedure of MoPn purification and infectivity determination was the same as previously described (11).

Infection

Mice were inoculated intranasally with various doses of MoPn in a volume of 40 μl. The mice were monitored daily for viability and weight changes following infection. As defined for individual experiments, mice were killed at selected days after infection, and lungs were aseptically isolated and homogenized in sucrose-phosphate-glutamic acid (SPG) buffer. Tissue homogenates were spun at 500 × g for 10 min at 4°C, and supernatants were kept at −70°C until tested.

Antibody measurement

Serum IgM, IgG1, IgG2a, and IgA Abs to MoPn EBs in each individual mouse were determined by ELISA as described (11). Results are expressed as ELISA titers at 60 min using the endpoint (cutoff at OD_{max} 0.5) of the titration curves compared with an internal standard (sera from C57BL/6 mice repeatedly infected with MoPn) run in each assay. Sera from naive C57BL/6 mice showed titers < 100 for IgM and < 50 for all other isotypes in this testing system. All serum samples were measured at least twice.

Determination of DTH responses

MoPn-specific DTH was measured as described (11). Mice were injected in the hind footpad with 25 μl of heat-inactivated MoPn EBs (5 × 10^6 inclusion-forming units (IFUs)) in one side and the same volume of SPG buffer in the other side. The difference in thickness between the two footpads at 48 and 72 h was used as a measure of the DTH response. No measurable difference was found between the two footpads in uninfected control mice.

Cytokine analysis of splenocytes

Spleen cell cytokine production was analyzed as described (11, 20). Mice were killed at various days following infection and spleen cells were cultured at 7.5 × 10^6 cells/ml with heat-inactivated MoPn EBs (1 × 10^3 IFU/ml) in the presence or absence of anti-CD4 (YTS-191) mAb (kindly provided by Dr. K. T. HayGlass, University of Manitoba, Winnipeg, Canada) at 5 μg/ml. Culture supernatants were collected at 72 and 96 h for cytokine determination. All cytokine determinations were done by ELISA as previously described (11). Purified capture and biotinylated detection Abs were purchased from PharMingen (San Diego, CA). IFN-γ levels in 72-h culture supernatants were tested by a two-mAb sandwich ELISA (XMG1.2 for capture and R4-6A2 for detection). IL-6 and IL-10 were tested using 96-h culture supernatants. IL-6 assays used MP5-20F3 as capture and mAb MP5-32C11 as detector Abs, respectively. IL-10 assays used JES-2A5 and SXC-1 as capture and detection Abs. Recombinant cytokine proteins (PharMingen) were used as standards in all the assays.

Statistics

Difference in mortality between wild-type and B cell-deficient mice were analyzed by the χ^2 test. Body weight loss and cytokine production were analyzed by Students’ t test. IFUs of MoPn in the infected lungs were transformed to base 10 logarithms and analyzed by Students’ t test.

Results

MoPn-infected B cell-deficient mice show significantly higher pulmonary MoPn growth and mortality than do wild-type mice

Wild-type and B cell-deficient mice were intranasally inoculated with 1 × 10^4 IFU of MoPn, and the viability of the mice was monitored daily until 30 days postinfection. As shown in Figure 1A, B cell-deficient mice showed early death starting at day 11 postinfection. Up to 30 days postinfection, most wild-type mice were still alive (the mortality rate was 19%), while 66% of the B cell-deficient mice had died. The difference in mortality between B cell-deficient and wild-type mice was statistically significant (p = 0.0003). Similar results were obtained when mice were infected with a lower dose of MoPn (4 × 10^3 IFU, Fig. 1B); the difference between wild-type and B cell-deficient mice in mortality was again statistically significant (p = 0.004). When the severity of infection was analyzed based on body weight loss, B cell-deficient mice...
showed earlier and greater body weight loss than wild-type mice. Since B cell-deficient mice started to die from day 11 (Fig. 1A) or day 12 (Fig. 1B) postinfection in the two separate experiments, the body weight changes shown in Figure 2 compare only the differences between wild-type and B cell-deficient mice in the period before any mouse death had occurred in the two experiments (days 11 and 12, respectively).

To further examine the in vivo growth of MoPn in the lung, mice were infected with a sublethal dose (1 × 10^3 IFUs) of MoPn. Table I shows four separate experiments in which mice were infected intranasally with 1 × 10^3 IFUs of MoPn and were killed at various days postinfection. B cell-deficient mice showed higher (10–1000-fold) in vivo growth of MoPn than did wild-type mice in all experiments testing chlamydial in vivo growth at days 7, 10, 15, and 17 postinfection. The differences were statistically significant in the one large experiment (8–9 mice/group) at day 17 postinfection (p = 0.0007). In aggregate, the data indicate that B cell-deficient mice are more susceptible to MoPn infection than wild-type mice as demonstrated by higher mortality rate, greater morbidity rate, and delayed clearance of MoPn infection from the lung.

**B cell-deficient mice show impaired DTH and Ab responses following MoPn infection**

We previously demonstrated that the DTH response to *C. trachomatis* is highly correlated with more rapid clearance of the organism in vivo and that the DTH response to *C. trachomatis* infection is entirely dependent on αβ T lymphocytes (11, 12). Since B cell-deficient mice showed considerably higher mortality and in vivo chlamydial growth (Fig. 1; Table I) we analyzed DTH responses in the B cell-deficient mice in comparison with wild-type control mice. Mice were intranasally infected with 1 × 10^3 IFUs of MoPn and *Chlamydia*-specific DTH responses were tested at day 13 postinfection. As shown in Figure 3, B cell-deficient mice showed significantly reduced DTH responses to MoPn than did wild-type mice (p = 0.009 at 48 h, and p = 0.047 at 72 h). Not surprisingly, Ab responses were completely abolished in μMT mice, confirming the B cell deficiency of these mice (Table II). Overall, the data show that B cells are necessary not only for producing Ab to *Chlamydia* but also for the initiation of efficient T cell responses (DTH) to pulmonary chlamydial infection.

**B cell-deficient mice fail to produce T cell cytokines following MoPn infection**

A consistent body of evidence demonstrates that Th1-like T cell responses and IFN-γ production are critical for clearance of *C. trachomatis* infection (11, 20–22) and that cytokine patterns of spleen cells are tightly associated with host susceptibility or resistance to chlamydial lung infection (11). The failure of B cell-deficient mice to mount efficient DTH responses suggests that T cell function in these mice is impaired or altered. To more directly examine T cell responses and cytokine production in B cell-deficient mice and their relationship with susceptibility to chlamydial infection, we analyzed the Ag-specific cytokine production of spleen cells in μMT mice following MoPn infection. As shown in Table III, splenocytes from infected B mice were intranasally infected with MoPn with 1 × 10^4 (A) or 4 × 10^3 (B) IFUs of MoPn, and body changes were measured following the infection. The results were presented as mean ± SEM.

![FIGURE 2.](image). Wild-type (○) and B cell-deficient (●) mice were intranasally infected with MoPn with 1 × 10^4 (A) or 4 × 10^3 (B) IFUs of MoPn, and body changes were measured following the infection. The results were presented as mean ± SEM. 

![FIGURE 3.](image). Wild-type (□) (8 mice/group) and μMT (●) (9 mice/group) mice were infected intranasally with 1 × 10^3 IFUs of MoPn and delayed-type hypersensitivity (DTH) were determined at 13 days following infection as described in Materials and Methods. Footpad swelling at 48 and 72 h was shown (mean ± SEM).
cell-deficient mice completely or partially failed to produce either Th1-related (IFN-γ) or Th2-related (IL-6 and IL-10) cytokines after in vitro Chlamydia-specific restimulation. Cytokine production, including IFN-γ, IL-6, and IL-10, in MoPn-infected wild-type mice was completely or largely dependent on CD4 T cells because anti-CD4 mAb (YTS-191) blocked the production of these cytokines (Ref. 12, Table III) while anti-CD8 mAb (YTS-169) failed to do so (data not shown). To examine whether the impairment of T cell response in μMT mice following MoPn infection is due to an intrinsic deficiency of T cells, we analyzed spleen T cell function in naive mice by testing its proliferative response and IFN-γ production following polyclonal T cell (Con A) stimulation. As shown in Table IV, spleen cells from μMT mice respond normally to Con A stimulation in terms of proliferation and cytokine production, suggesting that T cells were intact in μMT mice. In aggregate, the data show that the failure of B cell-deficient mice to generate efficient cell-mediated immune responses (DTH) to chlamydial infection is associated with the impaired cytokine, including IFN-γ, production by CD4 T cells following chlamydial infection.

We then examined whether naive B cells collected from uninfected mice can efficiently generate or reactivate T cell responses (IFN-γ production) in vitro after organism-specific restimulation. As shown in Table V, addition of naive B cells to the spleen cell culture of MoPn-infected μMT mice did not generate IFN-γ production in the presence of heat-inactivated MoPn stimulation. Similarly, addition of purified naive B cells to the culture of CD4-enriched spleen cells collected from MoPn-infected wild-type mice failed to significantly increase Ag-specific production of IFN-γ (Table V). The results suggest that the initial in vivo interaction between T cells and B cells is critical for T cell priming during chlamydial infection. Nevertheless, the mechanisms underlying this T-B cell interaction were not defined by the in vitro experiments.

### B cell-deficient mice that survived primary MoPn infection are not resistant to secondary chlamydial infection

As shown in Figure 1, about one-third of the B cell-deficient mice and >80% of wild-type mice survived primary infection. These mice recovered their original body weights within 1 month of the infection, suggesting the resolution of the primary infection. Previous studies showed that C57BL/6 mice completely clear lung infection within 1 month (11). To examine whether primary infection resulted in immunity, we reinfected the survivors of primarily infected B cell-deficient and wild-type mice with a relatively large dose of MoPn (1 × 10^4 IFU) to 47 to 50 days following primary infection. As controls, naive B cell-deficient and wild-type mice that were age-matched with the recovered mice were also infected in the same experiment. As shown in Figure 4, the primed wild-type survivors were immune to secondary infection as shown by negligible weight loss and 100% survival rate at 15 days following secondary infection. However, B cell-deficient mice that had recovered from previous chlamydial infection showed remarkably greater body weight loss and mortality (50%) over the same time period than primed wild-type survivors. The magnitude of body weight loss among primed B cell-deficient mice following challenge infection was similar to that of B cell-deficient mice that were infected for the first time, and the mortalities in these two groups were not statistically different (p = 0.24) (Fig. 4). The data demonstrate that B cell-deficient mice, unlike wild-type mice, do not develop acquired immunity to chlamydial infection and suggest that B cells are critical in host defense not only for

### Table II. Serum Ab titers (mean ± SEM, Log10) in wild-type and B cell-deficient (μMT) mice following C. trachomatis (MoPn) lung infection

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Day of Testing</th>
<th>IgM</th>
<th>IgG1</th>
<th>IgG2a</th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wild-type</td>
<td>μMT</td>
<td>Wild-type</td>
<td>μMT</td>
</tr>
<tr>
<td>1^a</td>
<td>16</td>
<td>5.5 ± 0.1</td>
<td>&lt;1</td>
<td>1.9 ± 0.0</td>
<td>&lt;1</td>
</tr>
<tr>
<td>2^b</td>
<td>33</td>
<td>5.3 ± 0.1</td>
<td>&lt;1</td>
<td>2.8 ± 0.1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>3^c</td>
<td>23</td>
<td>5.3 ± 0.1</td>
<td>&lt;1</td>
<td>3.0 ± 0.1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

^a Mice were infected with MoPn and bled at various days following infection. Sera collected individually (sera were not pooled) were tested for Ab production using ELISA as described in Materials and Methods. Normal sera from naive C57BL/6 mice were used as negative controls which showed titers <100 for IgM and <50 for all other isotypes.

^b Wild-type (eight mice/group) and μMT mice (nine mice/group) were infected with 1 × 10^3 IFUs of MoPn.

^c Wild-type and μMT mice were infected with 4 × 10^3 IFUs of MoPn (Fig. 1B). Ab responses in 10 survivors of wild-type mice and 4 survivors of μMT mice were measured.

### Table III. Splenic cell cytokine production (mean ± SEM) after Chlamydia-specific restimulation in wild-type and B cell-deficient (μMT) mice following C. trachomatis (MoPn) lung infection

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Day of Testing</th>
<th>Mice</th>
<th>No. of Mice</th>
<th>IFN-γ (U/ml)</th>
<th>IL-6 (pg/ml)</th>
<th>IL-10 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Anti-CD4 −</td>
<td>Anti-CD4 +</td>
<td>Anti-CD4 −</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>Wild-type</td>
<td>2</td>
<td>31 ± 10</td>
<td>1.0 ± 0.2</td>
<td>203 ± 35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>μMT</td>
<td>2</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>&lt;15</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>Wild-type</td>
<td>3</td>
<td>297 ± 98</td>
<td>0.2</td>
<td>891 ± 493</td>
</tr>
<tr>
<td></td>
<td></td>
<td>μMT</td>
<td>3</td>
<td>16 ± 16</td>
<td>&lt;0.2</td>
<td>96 ± 59</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>Wild-type</td>
<td>8</td>
<td>276 ± 63</td>
<td>ND</td>
<td>5037 ± 37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>μMT</td>
<td>9</td>
<td>3 ± 2</td>
<td>ND</td>
<td>211 ± 19</td>
</tr>
</tbody>
</table>

^a Mice were intranasally infected with 1 × 10^5 IFUs of MoPn and sacrificed at various days following infection. The spleen cells were cultured in the presence of heat-inactivated MoPn EBs, and the culture supernatants were measured for cytokine production as described in Materials and Methods.
primary infection but also for resistance to secondary chlamydial infection.

Discussion

This study demonstrates that B cell-deficient mice generated by μ-chain gene disruption fail to clear primary chlamydial lung infection and fail to establish immunity to reinfection. In addition to the absence of Ab responses, B cell-deficient mice also showed significantly impaired cell-mediated immune responses and Ag-specific T cell cytokine responses. The data indicate that B cells play an important role in host defense against C. trachomatis infection. Although the exact mechanism by which B cells play a role in host defense to chlamydial infection was not directly defined in this study, the data suggest that B cells may function in at least two ways: through Ab production; and in the initiation and effector phases of T cell responses to chlamydial infection.

The most evident function of B cells in immune response is the production of Abs to specific Ags. C. trachomatis has numerous Ags, including the major outer membrane protein, which are highly immunogenic in inducing Ab responses (14). Although Ab responses have been extensively studied, their role in protection against in vivo chlamydial infection remains unclear (13–15, 23–29). In vitro studies have clearly shown that Abs to specific chlamydial Ags such as the major outer membrane protein can neutralize chlamydial infectivity in cultured cell lines (23, 24). Some in vivo studies, including direct delivery of anti-chlamydial Abs and the hybridoma backpack model, also demonstrated measurable degrees of protection (25–27). However, serum Ab, especially IgG, responses have been poorly correlated with clearance of chlamydial infection in both animal models of infection (11, 15) and epidemiologic studies of affected humans (13, 14, 29). Nevertheless, none of these studies has answered the question as to whether B cells are essential in host defense against chlamydial infection. The data reported in the present study unambiguously demonstrate that gene knockout B cell-deficient mice have a serious defect in resistance to primary and secondary chlamydial lung infection and show that B cells are essential for host defense against C. trachomatis infection.

The necessity for B cells, however, may not simply mean the requirement for Abs for protective immunity to chlamydial infection. An interesting and probably more important observation from this study is the impairment of T cell responses to chlamydial Ags that is detected in B cell-deficient mice. The impairment of T cell responses is not due to intrinsic deficiency of T cells in B cell-deficient mice because the cells showed normal levels of proliferation and cytokine production to polyclonal (Con A) stimulation (Table IV). This finding of defective T cell response to chlamydial Ag in gene knockout B cell-deficient mice suggests that B cells play a crucial role in the initial phase of T cell activation following chlamydial infection.

It has long been documented that B cells can function as APCs in initiation of T cell responses (29–32). B cells that express Abs specific for an Ag are highly efficient for the presentation of that Ag to T cells, and the binding of Ag with its Ag receptor on B cells signals biologic changes in the class II presentation pathway of B cells (33). However, it is still debatable whether B cells are able to, or are essential for, priming of T cell responses in vivo. The available data are inconclusive with some studies showing failure of T cell responses and others showing normal T cell responses in B cell-deficient mice (31–39). This inconsistency may reflect differences in Ags tested and model systems used. Notably, reports of T cell function in gene knockout B cell-deficient mice have shown normal T cell responses to soluble protein Ags (36, 37). Unlike the protein Ags, however, chlamydial EBs express LPS and other non-LPS molecules in their outer membrane that are mitogenic for B lymphocytes (40, 41). More importantly, we have found that gene knockout mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>Cell</th>
<th>IFN-γ (U/ml) After In Vitro Stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (× 10^7/ml)</td>
<td>Medium</td>
</tr>
<tr>
<td>Wild-type</td>
<td>Whole spleen cell</td>
<td>5</td>
</tr>
<tr>
<td>Wild-type</td>
<td>CD4 T cell</td>
<td>3</td>
</tr>
<tr>
<td>Wild-type</td>
<td>Naive B cell</td>
<td>3</td>
</tr>
<tr>
<td>Wild-type</td>
<td>CD4 T cell + naive B cell</td>
<td>3</td>
</tr>
<tr>
<td>μMT</td>
<td>Whole spleen cell</td>
<td>5</td>
</tr>
<tr>
<td>μMT</td>
<td>Whole spleen cell + naive B cell</td>
<td>3</td>
</tr>
</tbody>
</table>

*Wild-type or B cell-deficient (μMT) mice (two mice/group) were infected intranasally with MoPn (1000 IFUs) and sacrificed at day 10 postinfection. Single-cell suspensions of whole spleen cell or CD4 T cell (prepared using a CD4 T cell column purchased from Biotex, Edmonton, Canada) were cultured with or without heat-inactivated MoPn in the presence or absence of purified naive B cells (prepared using a B cell column purchased from Biotex) from uninfected wild-type C57BL/6 mice. IFN-γ concentrations (mean ± SEM) in 72-h culture supernatants determined by ELISA.
deficient in T cells (αβ and γδ TCR) produce significant levels of IgM Ab following MoPn infection, which indicates the direct activation of B lymphocytes by chlamydia EBs (X. Yang and R. C. Brunham, unpublished data). It has been demonstrated that LPS-activated B cell blasts, but not resting B cells, can function as efficient APCs for T cell priming (39). The B cell-stimulating characteristics of \textit{C. trachomatis} imply that the organism may activate B cells in vivo on initial entry in the host, possibly enabling its Ags to be presented by activated B cell lymphoblasts. The observation we have made with \textit{C. trachomatis} are not without precedent. It was recently reported that B cell-deficient mice showed increased in vivo growth of another intracellular bacterial pathogen, \textit{Mycobacterium tuberculosis}, and exhibited diminished in vitro T cell-proliferative responses to mycobacterial Ag (42). We hypothesize that the ability of a pathogen or Ag to activate B cells early in an adaptive immune response may determine whether B cells play a role in the Ag presentation to and/or costimulation of T cells.

While this article was in preparation, Su et al. (43) reported that gene-disrupted B cell-deficient mice mounted normal levels of DTH response and cleared primary infection as efficiently as wild-type mice following genital MoPn infection. The reason for the differences between their findings and our data is not clear. However, the differences may be apparent than real since the two approaches differed in several respects. First, the studies differ in the chlamydial infection model used. We used a pulmonary infection model, and infection at this site appears to be a more sensitive test of immunity to chlamydial infection than does a genital infection model, possibly because MoPn lung infection induces more severe diseases. Such differences between lung and genital MoPn model infections were also observed when class I-deficient mice were evaluated (reviewed by Yang and Brunham (2)). Secondly, the times at which T cell responses were measured differ in the two studies. Su et al. (43) measured DTH and cytokine production after complete resolution of chlamydial infection (day 30 postinfection), while our study measured T cell responses in the early (days 7 and 10) and intermediate (day 17) phases of immune mediated clearance. Theoretically, measurement of T cell and cytokine responses during the process of infection should be more representative of the pattern of immune responses to chlamydial infection. In aggregate, the data from the two studies may suggest that B cell-deficient mice have delayed but not absent T cell responses. Such kinetic differences were also observed in lymphocytic choriomeningitis virus-infected B cell-deficient mice (44). Since high dose lung MoPn infection is lethal for B cell-deficient mice, such mice may not have sufficient time to generate and amplify efficient T cell responses through Ag presentation by non-B cell APCs. In contrast, chlamydial genital infection is relatively minor and B cell-deficient mice clearly survive. Therefore, T cell responses can reach normal level during the later stages in genital infection model via activation by other APCs. In line with this hypothesis,
B cell-deficient mice in our infection model system showed reduced but still measurable DTH responses, indicating that the B cell is not the only cell type capable of presenting chlamydial Ags to T cells. Finally, unlike the lung infection model, the chlamydial genital infection model requires the administration of medroxyprogesterone to enhance infection. How sex steroids influence the immune response to chlamydial infection is uncertain. However, recent studies have shown that progesterone favors the development of Th2-like responses and is suppressive for Th1-like responses (45–49). It is possible, therefore, that Th1-like responses such as DTH in the mice with genital chlamydial infection is lower than that in the mice with lung infection because progesterone was used for establishing genital infection. Consequently, in the genital infection model, the difference in DTH response between wild-type and μMT mice may not as obvious as that in the lung infection model. Clearly, more study is necessary to clearly elucidate the mechanisms underlying the surprising differences in B cell requirements for host defense during genital and lung chlamydial infection.

Since the two arms (humoral and cell-mediated) of immune responses are either completely or partially impaired in B cell-deficient mice, this study does not provide conclusive information regarding the relative importance of each effector mechanism in host defense against chlamydial infection. Rather, this study demonstrates the involvement of and the critical role for B cells in protective immunity to chlamydial infection. Moreover, the finding that B cells function in the initiation of T cell responses suggests that further efforts should focus on the role of B cells in the priming of T cells and their potential importance in host defense to infection with intracellular bacterial pathogens in general.

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References