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*J Immunol* published online 1 February 2010
http://www.jimmunol.org/content/early/2010/02/01/jimmunol.0901593
Mice Deficient in MyD88 Develop a Th2-Dominant Response and Severe Pathology in the Upper Genital Tract following Chlamydia muridarum Infection

Lili Chen,*† Lei Lei,* Xiaotong Chang,* Zhihong Li,* Chunxue Lu,*† Xiaoyun Zhang,* Yimou Wu, ‡ I-Tien Yeh,‡ and Guangming Zhong§

MyD88, a key adaptor molecule required for many innate immunity receptor-activated signaling pathways, was evaluated in a Chlamydia muridarum urogenital tract infection model. Compared with wild-type mice, MyD88 knockout (KO) mice failed to produce significant levels of inflammatory cytokines in the genital tract during the first week of chlamydial infection. MyD88 KO mice developed a Th2-dominant whereas wild-type mice developed a Th1/Th17-dominant immune response after chlamydial infection. Despite the insufficient production of early inflammatory cytokines and lack of Th1/Th17-dominant adaptive immunity, MyD88 KO mice appeared to be as resistant to chlamydial intravaginal infection as wild-type mice based on the number of live organisms recovered from vaginal samples. However, significantly high numbers of chlamydial organisms were detected in the upper genital tract tissues of MyD88 KO mice. Consequently, MyD88 KO mice developed more severe pathology in the upper genital tract. These results together have demonstrated that MyD88-dependent signaling pathway is not only required for inflammatory cytokine production in the early phase of host response to chlamydial infection but also plays a critical role in the development of Th1/Th17 adaptive immunity, both of which may be essential for limiting ascending infection and reducing pathology of the upper genital tract by chlamydial organisms. The Journal of Immunology, 2010, 184: 000–000.

Chlamydia pneumoniae and Chlamydia trachomatis are the two major chlamydial species that can affect human health. C. pneumoniae, by invading respiratory epithelial tissues, can cause various airway illnesses and is also associated with atherosclerosis (1, 2), whereas C. trachomatis, by invading human urogenital tract epithelial tissues, is a leading cause of sexually transmitted diseases, which can potentially result in severe complications including pelvic inflammatory diseases, ectopic pregnancy, and infertility in the affected women (3). Although Chlamydia-triggered inflammatory responses are thought to be largely responsible for Chlamydia-induced diseases (4), the precise pathogenic mechanisms remain unclear. A murine biovar of C. trachomatis (known as mouse pneumonitis [MoPn] agent and now classified as a new species, Chlamydia muridarum) has been used to study the mechanisms of C. trachomatis pathogenesis and immunity in a mouse genital infection model (5–9). Mice intravaginally infected with the C. muridarum organisms can develop inflammatory pathologies including endometritis and hydrosalpinx similar to those in humans (10). More importantly, studies with the C. muridarum urogenital tract infection mouse model have led to the conclusion that CD4+ T cell-dependent and IFN-γ-mediated immunity represents a major protection mechanism for controlling chlamydial infection (11). However, the same Th1 response may also contribute to the Chlamydia-induced inflammatory pathologies. Because MyD88 is a critical signaling adaptor molecule required by many pattern recognition receptors for initiating inflammatory responses (12) and may also directly or indirectly affect T cell responses during adaptive immunity (13–15), we evaluated the role of MyD88-mediated signaling in C. muridarum urogenital tract infection in the current study.

MyD88 is necessary for responses to all TLRs, except TLR3 and a subset of TLR4 signaling pathways, which are mediated by another adaptor molecule called TRIF (TOLL/IL-1R domain-containing adaptor-inducing IFN-β) (16–18). Although both MyD88- and TRIF-mediated signaling pathways are required for optimal detection of microbial infection (19, 20), different pathways may be preferred for dealing with different infections. Activation of TRIF-dependent pathways can lead to increased production of antiviral molecules (21), whereas activation of MyD88-dependent pathways can result in a wide spectrum of antimicrobial responses. For example, mice deficient in MyD88 but not TRIF were more susceptible to infection with nontypeable Haemophilus influenzae (22), Burkholderia pseudomallei (23), Pseudomonas aeruginosa (24), Brucella abortus (25, 26), Leishmania major (14), and Toxoplasma gondii (27). However, despite the diverse responses mediated by MyD88, MyD88 may not be always required for host defense. For example, host resistance to Staphylococcus aureus (24) and Aspergillus fumigatus (28) is independent of MyD88-mediated signaling pathways. Humans with MyD88 deficiency are more susceptible to pyogenic bacteria but not to other pathogens, and the increased susceptibility to the pyogenic bacterial infection improves with age (29). Thus, the role of MyD88 in host defense appears to depend on infection types and conditions.
Interestingly, infection dose may affect the role of MyD88 in *C. muridarum* infection. At a high infection dose, MyD88 was found to be critical in controlling the airway infection with *C. pneumoniae* because MyD88 knockout (KO) but not wild-type mice were unable to mount an effective early inflammatory response and displayed delayed clearance of the pathogens from the lung. As a result, the KO mice developed severe chronic inflammation in the lung with elevated toxic cytokines and increased mortality (30). However, when mice were infected at a lower dose of *C. pneumoniae*, the bacterial burden was lower in MyD88 KO than wild-type mice, presumably because of insufficient neutrophil infiltration to the site of infection for supporting *C. pneumoniae* replication as explained by the authors (31). Nevertheless, *C. pneumoniae*-induced inflammatory cytokine production seems to consistently depend on MyD88-mediated signaling pathways (11, 32, 33).

The roles of TLRs and MyD88 in *C. trachomatis* infection have also been studied. First, infection of oviduct epithelial cells with *C. muridarum* organisms can lead to upregulation of pattern recognition receptors (34). Second, *C. trachomatis*-induced cytokine production is largely dependent on MyD88 (35). Third, both TLRs and MyD88 can be recruited to the inclusion in the infected cells (36, 37). Finally, mice deficient in TLR2 cleared infection as efficiently as wild-type mice but developed less pathology in the oviduct than the wild-type mice (38), whereas there was a slight increase in recovery of live organisms from vaginal samples of MyD88 KO mice (35). The remaining questions are: can MyD88 deficiency lead to enhanced ascending infection to the upper genital tract? How does MyD88 deficiency affect the development of inflammatory pathologies in the upper genital tract? In the current study, we addressed these questions by carefully comparing chlamydial infection and pathologies in the upper genital tract as well as the host response phenotypes between MyD88 KO and wild-type mice. These studies are necessary because the role of MyD88 in host defense varies depending on infection types and conditions. These studies are medically significant because humans with MyD88 deficiency are known to be more susceptible to some microbial infection. We found that upon *C. muridarum* ure- genital tract infection, MyD88 KO mice failed to generate a rapid inflammatory response in the genital tract and developed a Th2- dominant adaptive immunity. Although both wild-type and MyD88 KO mice appeared to be similarly susceptible to chlamydial intravaginal infection based on live organisms recovered from vaginal swab samples, significantly higher numbers of chlamydial organisms were detected in the upper genital tract tissues of the MyD88 KO mice. Consequently, MyD88 KO mice also developed more severe pathology in the upper genital tract. Thus, MyD88, by both mediating the early-induced phase inflammation and ensuring the development of Th1/Th17-dominant adaptive immunity, plays a critical role in preventing chlamydial ascending infection and pathology in the upper genital tract.

### Materials and Methods

**Chlamydial organisms and infection**

The *C. muridarum* organisms (Nigg strain) were used to infect mice and mouse peritoneal macrophages (MDs) as well as HeLa cells (human cervical carcinoma epithelial cells; ATCC catalog number CCL2). The organisms were propagated, purified, aliquoted, and stored as described previously (39, 40). Female C57BL/6J mice wild type (stock number 000664; The Jackson Laboratory, Bar Harbor, ME) or with gene deficiency in MyD88 (a gift from Dr. S. Akira, Osaka University, Osaka, Japan) were used at the age of 7–8 wk old. Each mouse was inoculated intravaginally with 1 × 10^5 inclusion forming units (IFUs) of live *C. muridarum* organisms in 20 μl sucrose–phosphate–glutamate (SPG) buffer consisting of 218 mM sucrose, 3.76 mM KH₂PO₄, 7.1 mM K₂HPO₄, and 4.9 mM glutamate (pH 7.2)). Five days prior to infection, each mouse was injected with 2.5 mg Depo-provera (Pharmacia Upjohn, Kalanamooz, MI) s.c. to synchronize estrous cycle and increase mouse susceptibility to chlamydial infection. For some mice, a secondary infection was similarly carried out on day 54 after primary infection. Depo-provera was also applied to the mice 5 d prior to the secondary infection. For in vitro infection of MDs, mouse MDs were collected from peritoneal cavity as described previously (41). Briefly, the mouse peritoneal cavity was injected with 4–5 ml cold Hanks’ buffer (2.5 mM HEPES [pH 7.4], 140 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM NaHPO₄, 25 mM glucose, and 0.05% BSA) using a 27-gauge needle. After gentle massage, the solution was slowly withdrawn from the mouse peritoneal cavity using a 20-gauge needle. After counting the total number of viable cells, the peritoneal cavity-derived cells were resuspended in RPMI 1640 with 10% FCS and 10 μg/ml gentamicin, and 2 × 10⁶ cells were added to each well of 24-well plates. The plates were incubated at 37°C for 2 h to allow MDs to adhere. After washing away nonadherent cells, fresh medium was added into each well for continuing incubation. The adherent MDs were cultured overnight prior to chlamydial inoculation. Chlamydial organisms diluted in cell growth medium were directly inoculated onto the cell monolayers at a multiplicity of infection of 5. The infected cultures were incubated for 24 h at 37°C in a CO₂ incubator before harvesting for cytokine measurement by ELISA. To infect HeLa cells, HeLa cells grown on coverslips in 24-well plates were infected with *C. muridarum* organisms as described previously (39, 40). The infected cultures were processed for immunofluorescence assay as described below.

**Titrating live chlamydial organisms from vaginal swabs and cervix, uterine horn, and oviduct tissue homogenates**

To monitor live organism shedding, vaginal swabs were taken on different days after the intravaginal infection (once every 3–4 d). Each swab was dissolved in 500 μl of SPG, followed by sonication on ice. In some experiments, mice were sacrificed, and genital tissues were sterilely isolated and separated into three portions including cervix, uterine horn, and oviduct (containing ovary). Each tissue sample was homogenized in 400 μl SPG, followed by a brief sonication on ice. The released organisms from either the swab or tissue homogenate samples were immediately titrated on HeLa cell monolayers in duplicates as described previously (42). Briefly, serially diluted samples were inoculated onto HeLa cell monolayers grown on coverslips in 24-well plates. After incubation for 24 h in the presence of 2 μg/ml cycloheximide, the cultures were processed for immunofluorescence assay as described below. The inoculations were counted under a fluorescence microscope. Five random fields were counted per coverslip. For coverslips with <1 IFU/field, the entire coverslips were counted. Coverslips showing obvious cytotoxicity were not taken into count. The total number of IFUs per sample was calculated based on the number of IFUs per field, number of fields per coverslip, dilution factors, and inoculation and total sample volumes. An average was taken from the serially diluted and duplicate samples for any given swab. The calculated total number of IFUs per swab was converted into log₁₀, and the log₁₀ IFUs were used to calculate means and SD for each group at each time point.

**Evaluating pathology and detecting chlamydial Ags in mouse genital tissues**

Mice were sacrificed on different days after primary infection (some mice sacrificed on day 80 were reinfected on day 54), and the mouse urogenital tract tissues were isolated. Before the tissues were removed, an in situ gross examination was performed for evidence of macroscopic abnormalities. The tissues were then fixed in 10% neutral formalin and embedded in paraffin and serially sectioned longitudinally (with 5 μm/each section). Efforts were made to include cervix, both uterine horns, and oviducts as well as luminal structures of each tissue in each section. For evaluating inflammation, the sections were stained with H&E as described elsewhere (10). The H&E-stained sections were assessed (by S.Y.) and a 1–4 grading system was scored for severity of inflammation and pathologies based on the modified schemes established previously (10, 42, 43). The uterine horns and fallopian tubes were scored separately. Scoring for dilatation of uterine horn or fallopian tube: 0, no significant dilatation; 1, mild dilatation of a single cross-section; 2, one to three dilated cross-sections; 3, more than three dilated cross sections; and 4, confluent pronounced dilatation. Scoring for inflammatory cell infiltration: 0, no infiltration; 1, infiltration of single cells; 2, infiltration at two to four foci; 3, infiltration at more than four foci; and 4, confluent infiltration. Scores assigned to...
individual mice were calculated into means ± SEs for each group of animals. For detecting chlamydial Ags, five sections were collected from each genital tissue block with an interval of five cuts between each section. After deparaffinization and Ag retrieval treatments, the tissue section slides were blocked with 10% goat serum (catalog number 200-6210; Life Technologies, Grand Island, NY) in PBS for 30 min at room temperature. A rabbit anti-MoPn antiserum diluted at 1/5000 in PBS containing 1% BSA (G. Zhong, unpublished data) was applied to the slides followed by a Cy2-conjugated goat anti-rabbit IgG (green) (Jackson ImmunoResearch Laboratories, West Grove, PA) together with Hoechst dye (blue) (Sigma-Aldrich, St. Louis, MO). The slides were read using a fluorescence microscope as described below. Besides taking images of representative views for different genital tissue sections, all five slides from each tissue were counted for chlamydial inclusions across the entire sections by first screening the slides under a low-power objective lens and confirming the authenticity of inclusions under a high-power objective lens. Finally, the number of inclusions per section was calculated for each mouse tissue sample.

**Immunofluorescence assay**

HeLa cells grown on coverslips were fixed with 2% paraformaldehyde (Sigma-Aldrich) dissolved in PBS for 30 min at room temperature, followed by permeabilization with 2% saponin (Sigma-Aldrich) for an additional 1 h. After washing and blocking, the cell samples were subjected to Ab and chemical staining. Hoechst (blue) (Sigma-Aldrich) was used to visualize nuclear DNA. For titrating IFUs from mouse vaginal swab samples, a mouse anti-chlamydia LPS Ab (catalog number MB597H) (G. Zhong, unpublished observation) plus a goat anti-mouse IgG conjugated with Cy3 (red) (Jackson ImmunoResearch Laboratories) were used to visualize chlamydial inclusions. For titrating and isotyping mouse antisera, *C. muridarum*-infected HeLa cells grown on coverslips were processed as above. The serially diluted mouse antisera were used as the primary Abs. The primary Abs were detected with the fluorescein-conjugated goat Abs against mouse total IgG, IgG1, IgG2a, IgG2b, and IgG2c (Jackson ImmunoResearch Laboratories). The detection Ab alone-labeled samples were used as controls. The coverslips were observed under an Olympus AX-70 fluorescence microscope equipped with multiple filter sets and a Hamamatsu charge-coupled device camera (Olympus, Melville, NY).

**ELISA**

Cytokines from mouse vaginal swab samples, Mφ culture supernatants and mouse spleen cell restimulation culture supernatants were measured using commercially available ELISA kits. The kits for mouse IL-1α (catalog number DY400), IL-6 (catalog number DY406), TNF-α (catalog number DY410), MIP-2 (mouse homolog of human IL-8, DY452), IFN-γ (catalog number DY485), IL-17 (catalog number M1700), IL-4 (catalog number DY421), and IL-5 (catalog number DY465) were all obtained from R&D Systems (Minneapolis, MN). The ELISA was performed following the instructions provided by the manufacturer or described elsewhere (44–46). Briefly, 96-well ELISA microplates (Nalge Nunc International, Rochester, NY) were coated with a capture Ab and after blocking, the cytokine samples or standards were added to the coated plates followed by a biotin-conjugated detection Ab. The Ab binding was detected with a HRP-conjugated Avidin plus a soluble colorimetric substrate (ABTS). The absorbance was taken at 405 nm using a microplate reader (Molecular Devices, Sunnyvale, CA). The cytokine concentrations were calculated based on absorbance values, cytokine standards, and sample dilution factors and expressed as picograms or nanograms per milliliter.

To prepare vaginal swab samples for cytokine measurements, each mouse vaginal swab was sonicated in 0.5 ml SPG on ice as described above. After a portion was used for titrating live organisms, the rest vaginal swab samples were stored at −80°C as source materials for cytokine measurements. The frozen vaginal swab samples were completely thawed on ice and mixed well before dilution. Both neat and serially diluted vaginal swab samples were applied to the corresponding ELISA plate wells with 50 μl/well. Pilot experiments have shown that the SPG solution used for sonicating vaginal swab samples does not interfere with cytokine binding to the capture Abs coated in ELISA plates. The Mφ cultures were set up and infected with MoPn organisms as described above, and the supernatants were collected and applied to the ELISA plates with or without dilution. To prepare mouse spleen cell in vitro restimulation cultures, splenocytes were harvested from mice infected with MoPn on day 80 after the primary infection (some groups of mice were also reinfected on day 54) and cultured in 96 well plates at a density of 5 × 10^6 cells/well with or without stimulation with UV-inactivated MoPn EBs at a final concentration of 1 × 10^6 IFU/ml. After culturing for 3 days, the culture supernatants were collected for cytokine measurements with or without dilution.

**Statistical analysis**

ANOVA Test (www.physics.csbsju.edu/stats/anova.html) was performed to analyze the IFU and cytokine measurement data from multiple groups and a two-tailed Student *t* test (Microsoft Excel) to compare two given groups. The semiquantitative pathology score data were analyzed with Student *t* test or Wilcoxon-signed rank test (an alternative nonparametric method of *t* test; http://faculty.vassar.edu/lowry/wilcoxon.html). The Fisher’s exact test (www.danielsoper.com/statcalc2/calc29.aspx) was used to analyze the data on incidence of uterine dilation and oviduct hydrosalpinx shown in Table 1.

**Results**

*Mice deficient in MyD88 not only failed to produce significant levels of early inflammatory cytokines but also developed a Th2-dominant adaptive response following urogenital tract infection with C. muridarum organisms*

Intravaginal infection with *C. muridarum*-induced production of inflammatory cytokines IL-1α, IL-6, and TNF-α as well as chemokine MIP-2 in the urogenital tract of wild-type C57 mice starting as early as day 3 and reaching the peak on day 7 postinfection (Fig. 1). This cytokine production kinetics is consistent with those reported in...
production in the urogenital tract of MyD88 KO mice is due to the inability of mouse genital tract cells to respond to chlamydial infection.

Because MyD88-mediated signaling is also known to impact adaptive immunity (13–15), we next compared the adaptive immune responses between wild-type and MyD88 KO mice after chlamydial infection. Mice were inoculated intravaginally with *C. muridarum* organisms, and on day 80 postinfection, mouse splenocytes were harvested and restimulated with UV-inactivated MoPn organisms. Some mice were reinfected with MoPn organisms on day 54. As shown in Fig. 3, we found that splenocytes from wild-type C57 mice either with primary infection alone or after secondary infection produced high levels of IFN-γ but low levels of IL-4 and IL-5, indicating that the wild-type mice developed a Th1-biased immune response, which is consistent with what was described previously (48). We also found that the wild-type C57 mice developed a robust Th17 response because the splenocytes also produced a high level of IL-17 upon restimulation with chlamydial Ags. However, splenocytes from the MyD88 KO mice produced only minimal levels of IFN-γ or IL-17 but significantly high levels of IL-4 and IL-5, indicating that

**FIGURE 3.** Effect of MyD88 deficiency on isotype switch of chlamydial Ag-specific IgG Abs following intravaginal infection with *C. muridarum*. Both wild-type (●, n = 9 for either primary [I'] infection alone or with reinfection [II'] groups in experiment 1 [Exp 1]; n = 12 with I' infection only in experiment 2 [Exp 2]) and MyD88 KO (●, n = 10 in Exp 1; n = 12 in Exp 2) mice were infected with *C. muridarum* organisms on day 80 postinfection. A, The titers of *Chlamydia*-specific total IgG Abs and IgG2b in different isotypes (including IgG1, IgG2a, and IgG2c) were determined using an immunofluorescence assay with *C. muridarum*-infected HeLa cells as Ags. The highest dilution of a given mouse serum that still positively labeled chlamydial Ags was determined as the titer of that serum sample. The Ab titers were expressed as log10 dilution. Data shown was from Exp 1, and similar results were obtained from Exp 2 (data not shown). B, The ratio of IgG2a versus IgG1 was calculated for each mouse group. Note that the MyD88 KO mice from both experiments 1 and 2 produced less IgG2a and more IgG1, indicative of Th2-dominant responses (+p < 0.05; **p < 0.01; Student t test).
Deficiency in MyD88 allows chlamydial organisms to ascend into the upper genital tract following intravaginal infection

To evaluate the role of MyD88-mediated signaling pathways in chlamydial infection, we first compared the levels of live organisms shedding from the genital tract between wild-type and MyD88 KO mice (Fig. 5), because this is a most commonly used method for monitoring chlamydial infection in mice (35, 40, 49). MyD88 KO mice only displayed a slightly increased shedding intensity and duration, which is consistent with a previous observation (35) but still surprising to us given the severe deficiency in early inflammatory response but also necessary for developing Th1/17-dominant adaptive immunity against chlamydial urogenital infection.

Because live organism shedding detected in the vaginal swabs may only reflect the level of chlamydial infection in the lower genital tract, we further measured the live organisms in mouse genital tissue homogenates following chlamydial intravaginal infection (Fig. 6). We found that genital tissues from both wild-type and MyD88 KO mice contained similar amounts of live organisms on day 10. However, by day 23, live organisms were detected in the upper genital tract tissues (uterine horn and oviduct) of the MyD88 KO but not wild-type mice, suggesting that more chlamydial organisms ascended into uterine horn and oviduct tissues of the MyD88 KO mice. This conclusion is further supported by direct detection of chlamydial Ags in mouse genital tissue sections (Fig. 7). There were significantly higher numbers of chlamydial inclusions in the cervix, uterine horn, and oviduct tissue sections of MyD88 KO than wild-type mice on day 10 postinfection, which was surprising to us because the IFUs recovered from these tissue samples were similar to those detected in wild-type mice.
homogenates were similar between wild-type and MyD88 KO mice. This observation may suggest that not all inclusions detected in the tissue sections contain infectious organisms. More importantly, by day 23, chlamydial inclusions were no longer detected in any tissue sections of wild-type mice, but there were still significant numbers of chlamydial inclusions in all tissue sections of the MyD88 KO mice. These observations together demonstrate that chlamydial organisms ascend to and replicate more efficiently in the upper genital tract tissues of MyD88 KO mice.

**Mice deficient in MyD88 developed more severe inflammatory pathology in the upper genital tract after intravaginal infection with C. muridarum**

Because MyD88 KO mice permitted more obvious chlamydial ascending infection, we further evaluated whether MyD88 KO mice also developed more severe pathology in the upper genital tract. Groups of mice that were monitored for live organisms shedding from vaginal samples as described above were sacrificed on day 80 after primary infection (a secondary infection was applied on day 54 to half of the mice in experiment 1 but no secondary infection in experiment 2), and the genital tract tissues were examined for inflammatory pathologies both in gross appearance (Fig. 8, Table I) and under microscope (Fig. 9). Uterine horn dilatation and oviduct hydrosalpinx are hallmarks of the urogenital tract pathologies caused by chlamydial vaginal infection in mice (10). MyD88 KO mice displayed significantly higher rates of hydrosalpinx (experiment 1), and both uterine horn dilatation and hydrosalpinx (experiment 2) caused by primary infection (Fig. 8, Table I). In experiment 1, 6 of the 10 MyD88 KO mice with primary infection alone displayed bilateral hydrosalpinx, and an additional 3 mice displayed unilateral hydrosalpinx. Only one MyD88 KO mouse lacked any obvious sign of hydrosalpinx. In contrast, only one and three of the nine wild-type mice with primary infection alone developed bilateral and unilateral hydrosalpinx respectively (no hydrosalpinx in the rest five mice). Incidence of bilateral hydrosalpinx in MyD88 KO mice is significantly higher than that of the wild-type mice (p < 0.05). However, the difference disappeared after a secondary infection. In experiment 2, 9 of the 12 MyD88 KO mice with primary infection displayed bilateral hydrosalpinx, whereas only 2 of the 12 wild-type mice developed bilateral hydrosalpinx. The incidence of bilateral hydrosalpinx in MyD88 KO mice is significantly higher than that of the wild-type mice (p < 0.05). Furthermore, the incidence of bilateral uterine horn dilatation in the MyD88 KO group is also significantly higher than that of the wild-type mouse group (p < 0.05).

We next semiquantitatively analyzed the lumen dilatation and inflammatory infiltration under microscope. The results from experiment 1 were presented in Fig. 9. We found that the MyD88 KO mice (after primary infection alone) displayed significantly higher scores in both inflammation and lumen dilatation of the oviduct tissues compared with the wild-type mice (p < 0.01), which supports the gross appearance observation. Similar results were obtained from experiment 2 (data not shown). Thus, the results from two independent experiments have consistently demonstrated that MyD88 deficiency can lead to more severe pathologies in mouse upper genital tract after C. muridarum intravaginal infection.

**Discussion**

Although it is well established that a Th1-dominant adaptive response plays a critical role in protection against chlamydial infection (11), the role of innate immunity components in host defense and pathogenesis during chlamydial urogenital infection has not been well elucidated. MyD88 is a key adaptor molecule in innate immunity. In the current study, we have demonstrated that MyD88 plays a critical role in host defense against chlamydial urogenital infection by both mediating the early-induced inflammatory...
responses and steering the adaptive immunity toward Th1 phenotype. First, MyD88 KO mice failed to produce significant levels of inflammatory cytokines in the genital tract upon intravaginal infection with *C. muridarum* organisms. Second, Mfs from MyD88 KO mice failed to respond to *C. muridarum* infection for cytokine production. Third, splenocytes from *Chlamydia*-infected MyD88 KO mice produced high levels of Th2 cytokines IL-4 and IL-5 but only minimal levels of Th1 cytokine IFN-γ and Th17 cytokine IL-17. Fourth, the *Chlamydia*-specific IgG2a/IgG1 ratio was <1 (indicative of a Th2-dominant response) in MyD88 KO mice. Fifth, more chlamydial organisms ascended into upper genital tract of the MyD88 KO mice. Finally, the MyD88 KO mice developed significantly more severe hydrosalpinx and inflammatory infiltration in the oviduct. Clearly, MyD88 is essential for controlling chlamydial ascending infection and reducing *Chlamydia*-induced pathology in the upper genital tract.

Table 1. Incidence of gross pathologies

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<th>Primer I</th>
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Mice were sacrificed after primary infection (I°) alone or plus secondary (II°) infection, and the reproductive tissues were isolated and inspected for gross pathologies including uterine horn dilatation and oviduct hydrosalpinx (see Fig. 8 for images from Exp 1). Number of mice showing pathology on single or both sides of the reproductive tissues were recorded and tabulated. The incidence of bilateral hydrosalpinx in MyD88 KO mice after primary infection alone is significantly higher than that of wt mice in Exp 1 (*p < 0.05, two-tailed Fisher’s exact test). In Exp 2, the rates of both bilateral hydrosalpinx and uterine horn dilatation in MyD88 KO mice are significantly higher than those of wt mice (*p < 0.05). Exp, experiment; wt, wild-type.
Although MyD88 is required for signaling pathways of most TLRs as well as IL-1R (16–18), our current finding suggests that chlamydial infection can induce pathology in the absence of MyD88 because mice deficient in MyD88 developed more severe inflammatory pathology in the upper genital tract. However, various previous studies have shown that mice deficient in either TLR-2 (38) or caspase-1 (40) displayed significantly reduced levels of oviduct pathology, suggesting that inflammation triggered by TLR2 or participated by IL-1β can contribute to Chlamydia-induced pathology. The seemingly conflicting phenotypes can be explained as follows. Lack of MyD88 represents a significant deficiency in host defense against chlamydial infection, leading to increased ascending infection. The prolonged presence of chlamydial Ags in mouse upper genital tract may result in activation of MyD88-independent pathways for producing tissue-damaging cytokines. This hypothesis is supported by the fact that many cytokine, such as IFN-β, inflammatory protein-10, MCP-1, and Rantes, can be produced in MyD88-independent manner (21). In addition, it has been shown that Chlamydia-induced Th2-dominant responses can contribute to chlamydial pathogenesis in susceptible mice (50, 51). Thus, both persistent Ag stimulation (as a result of the increased ascending infection) and pathogenic Th2 responses can contribute to the exacerbated oviduct pathologies in the MyD88 KO mice. In contrast, deficiency in a single receptor such as TLR2 or a single cytokine such as IL-1β may only represent a limited (although important) deficiency in host defense against chlamydial infection. These gene-deficient mice may not develop as severe ascending infection as MyD88 KO mice do. Thus, lack of either TLR2 (38) or caspase-1 (40) only led to the attenuation of Chlamydia-induced inflammatory pathology without significantly compromising host defense to chlamydial infection. It seems to be a common theme that immune component deficiency that does not significantly affect chlamydial infection often results in attenuated pathology in the upper genital tract, whereas deficiency that significantly reduces host defense against chlamydial infection can lead to exacerbated pathology. For example, CD40-mediated costimulation is more important than CD28 costimulation in host defense against chlamydial infection. Mice deficient in CD28 displayed reduced oviduct pathology, whereas mice deficient in CD40L developed more severe pathology after chlamydial infection (47).

The observation that MyD88 KO mice developed serious ascending infection and severe pathologies in the upper genital tract suggests that MyD88-dependent responses are protective against both chlamydial infection and pathology. It will be interesting to investigate whether humans with loss of function mutations in MyD88-mediated signaling pathways are more susceptible to tubal pathology after C. trachomatis infection. In contrast, further identification of the chlamydial ligands that can activate MyD88 signaling pathways may provide useful information for developing chlamydial vaccines.

Disclosures
The authors have no financial conflicts of interest.
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