

MyD88 Deficiency Leads to Decreased NK Cell Gamma Interferon Production and T Cell Recruitment during *Chlamydia muridarum* Genital Tract Infection, but a Predominant Th1 Response and Enhanced Monocytic Inflammation Are Associated with Infection Resolution[▽]

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We have previously shown that MyD88 knockout (KO) mice exhibit delayed clearance of *Chlamydia muridarum* genital infection compared to wild-type (WT) mice. A blunted Th1 response and ineffective suppression of the Th2 response were also observed in MyD88 KO mice. The goal of the present study was to investigate specific mechanisms whereby absence of MyD88 leads to these effects and address the compensatory mechanisms in the genital tract that ultimately clear infection in the absence of MyD88. It was observed that NK cells recruited to the genital tract in MyD88 KO mice failed to produce gamma interferon (IFN- γ) mRNA and protein. This defect was associated with decreased local production of interleukin-17 (IL-17), IL-18, and tumor necrosis factor alpha (TNF- α) but normal levels of IL-12p70. Additionally, recruitment of CD4 T cells to the genital tract was reduced in MyD88 KO mice compared to that in WT mice. Although chronic infection in MyD88 KO mice resulted in oviduct pathology comparable to that of WT mice, increased histiocytic inflammation was observed in the uterine horns. This was associated with increased CCL2 levels and recruitment of macrophages as a potential compensatory mechanism. Further deletion of TLR4-TRIF signaling in MyD88 KO mice, using TLR4/MyD88 double-KO mice, did not further compromise host defense against chlamydiae, suggesting that compensatory mechanisms are Toll-like receptor (TLR) independent. Despite some polarization toward a Th2 response, a Th1 response remained predominant in the absence of MyD88, and it provided equivalent protection against a secondary infection as observed in WT mice.

Chlamydia trachomatis is a common sexually transmitted bacterial pathogen that can cause oviduct inflammation and subsequent infertility. Chlamydial clearance is dependent on the generation of gamma interferon (IFN- γ)-producing CD4⁺ Th1 cells. Studies in animal models and humans reveal a direct correlation between the strength of the chlamydia-specific Th1 immune response and resistance to infection and disease (4, 6, 18, 21, 27).

Chlamydiae interact with multiple host pathogen recognition receptors (PRRs), including cell membrane Toll-like receptors (TLRs) and intracellular cytosolic PRRs (8, 23, 29). We found that TLR2 is a key receptor involved in chlamydial recognition as demonstrated by the detection of significantly

reduced levels of tumor necrosis factor alpha (TNF- α), interleukin-6 (IL-6), and macrophage inflammatory protein 2 (MIP-2) (CXCL2) in TLR2 knockout (KO) fibroblasts and macrophages infected *in vitro* with *Chlamydia muridarum* (8). Additionally, TLR2 was found to traffic to the chlamydial inclusion in infected cells (22). The role for TLR4 is less apparent, although TLR4 KO fibroblasts and macrophages infected with *C. muridarum* displayed an increased release of inflammatory cytokines, suggesting that chlamydial recognition by TLR4 may down-modulate signaling initiated by other receptors (8). We also determined that in the absence of MyD88, an adaptor molecule downstream of both TLR2 and TLR4, TNF- α production was significantly compromised in infected macrophages (19). Chlamydiae are also recognized by cytosolic PRRs that act independently of MyD88, including nucleotide-binding oligomerization domain 1 (NOD1), which plays a role in chlamydia-induced NF- κ B activation (29), and stimulator of interferon gene (STING) protein, important for chlamydial induction of type I interferon (23).

Examination of chlamydial infection in mice genetically deficient in these various PRRs has revealed a partial redundancy in chlamydial activation of the transcription factor NF- κ B and the production of inflammatory chemokines and cytokines required for recruitment and activation of effector T cells. For

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example, although mice deficient in TLR2 exhibit decreased proinflammatory cytokine responses, these mice resolve *C. muridarum* infection normally and are protected from chronic oviduct pathology (8). These data demonstrate that alternative PRR signaling pathways are active in promoting an effective adaptive immune response (8). Although a phenotype was not detected during *in vivo* infection of TLR4 KO mice (8), a role for TLR4 might be more evident in the absence of other PRR signaling pathways. Despite the demonstration of a role for NOD1 in chlamydia-induced NF- κ B activation during *in vitro* infection, *in vivo* deficiency of NOD1 did not alter the course of murine chlamydial genital tract infection or resulting pathology (29). Despite this redundancy in PRR recognition of chlamydiae, we had previously shown that MyD88 KO mice exhibit delayed clearance of *C. muridarum* genital infection compared to wild-type (WT) mice (19), suggesting that multiple PRRs that signal through MyD88 could recognize *C. muridarum*. Alternatively, independently of TLR signaling, MyD88 could be necessary for generation of an optimal Th1 response. Indeed, in a recent work by Chen et al. (3), it was demonstrated that MyD88 KO mice exhibit responses suggestive of a Th2 response, including the detection of serum IgG1 and splenocyte production of IL-4.

In this study, our primary goal was to determine the cellular mechanisms dependent on MyD88 that are necessary for optimal resolution of *C. muridarum* genital tract infection. In addition, we sought to determine the role of the TLR4-TRIF pathway and MyD88-independent residual/compensatory responses operative in eventual resolution of infection in the absence of MyD88.

MATERIALS AND METHODS

Chlamydial stocks. *Chlamydia muridarum* strain Nigg was grown in mycoplasma-free McCoy cells as described previously (2, 8). Elementary bodies were harvested from infected cells, resuspended in SPG buffer (250 mM sucrose, 10 mM sodium phosphate, and 5 mM L-glutamic acid, pH 7.2), and quantified as inclusion-forming units (IFU) on McCoy cell monolayers as described previously (2).

Animals. MyD88 gene KO mice or *myd88*^{-/-} mice (originally from S. Akira) (1) were purchased from the European Mouse Mutant Archive, Neuberger/Munich, Germany, and have been previously backcrossed with C57BL/6 mice over 10 generations (10, 13). TLR4^{ps-del} (*tlr4*^{-/-}) mice have a deletion in the *tlr4* gene (C57BL/10SCN) and were obtained from the Jackson Laboratory. Mice deficient in MyD88 and TLR4, TLR4/MyD88 double-knockout (DKO) mice, were generated by crossing F1 progeny of TLR4 KO mice and MyD88 KO mice (24). Age-matched (8 to 12 weeks) C57BL/6 female mice were used as controls for all experiments. Mice were given food and water *ad libitum* in an environmentally controlled room with 12 h of light and dark cycles, respectively. All animal experiments were preapproved by the Institutional Animal Care and Use Committee of the University of Arkansas for Medical Sciences.

Murine genital infection with *C. muridarum* and analysis of cytokines in genital secretions and cervical homogenates. Mice received 2.5 mg of medroxyprogesterone acetate (Depo-Provera; Upjohn) in 0.1 ml of saline subcutaneously (s.c.) 7 days before vaginal infection. Mice were anesthetized using sodium pentobarbital and infected by placing *C. muridarum* (3×10^5 IFU in 30 μ l SPG buffer) into the vaginal vault. Mice were monitored by swabbing the vaginal vault and cervix with a calcium alginate swab (Spectrum Medical Industries) at various times following infection, and the bacterial burden was determined by enumerating IFU using McCoy cells (14). The number of inclusion bodies within 20 fields (40 $\times$) was counted using an Olympus fluorescence microscope, and IFU were calculated. To assess bacterial burden upon challenge infection, WT, MyD88 KO, and TLR4/MyD88 DKO mice administered medroxyprogesterone acetate 96 days post-primary infection were reinoculated intravaginally with 3×10^5 IFU of *C. muridarum* on day 104. Two challenge experiments were conducted. In the first, 0.3 mg doxycycline in 0.1 ml phosphate-buffered saline (PBS) was administered intraperitoneally (i.p.) on days 97 to 99 to ensure that infection

had completely resolved in the genetically deficient mice; in the second experiment, no doxycycline was administered prior to reinoculation. Genital tract secretions were collected as described previously (7). Cytokine levels in individual genital tract sponge eluates were determined using enzyme-linked immunosorbent assay (ELISA) kits from R&D Systems or the Milliplex bead array (Millipore). To make cellular lysates from cervical tissues, tissues from infected mice were minced and homogenized in 500 μ l of buffer containing 50 mM Tris, 150 mM NaCl, 1% NP-40, and a protease inhibitor cocktail (Pierce). Homogenized tissues were sonicated and centrifuged at $9,300 \times g$ at 4°C to remove cellular and nuclear debris, and 50 to 100 μ l of supernatant was used for ELISA. Oviducts from infected mice at day 14 postinfection were processed by mincing the tissue in RPMI and passing it through a 70- μ m nylon cell strainer (BD Falcon). Cells were resuspended in 1 ml of RPMI, from which 800 μ l was processed for flow cytometry. One-half of the remaining cells were treated with protease inhibitors, lysed by two freeze-thaw cycles, cleared by centrifugation at $9,300 \times g$ at 4°C, and used for the Milliplex cytokine array. The remaining cells were lysed by freeze-thaw cycles, sonicated for a minute, centrifuged at $100 \times g$ at 4°C, and processed for IFU enumeration.

Histopathology. Genital tract tissues were extracted *en bloc* from mice sacrificed on day 7, 42, or 56 post-primary infection or day 56 postchallenge. Tissues were fixed in 10% buffered formalin and embedded in paraffin. Longitudinal sections (4 μ m) were stained with hematoxylin and eosin and evaluated by a pathologist blinded to the experimental design. Each anatomic site (exocervix, endocervix, uterine horn, oviduct, and mesosalpinx) was assessed independently for the presence of neutrophils, mononuclear cells (lymphocytes and monocytes), plasma cells, and fibrosis. Right and left uterine horns and right and left oviducts were evaluated individually. A four-tiered semiquantitative scoring system was used to quantitate the inflammation and fibrosis: 0, normal; 1+, rare foci (minimal presence) of parameter; 2+, scattered (1 to 4) aggregates or mild diffuse increase in parameter; 3+, numerous aggregates (>4) or moderate diffuse or confluent areas of parameter; 4+, severe diffuse infiltration or confluence of parameter. Luminal distention of the uterine horns, granulomas, and dilatation of the oviducts were graded from 1 to 4, with grade 4 representing peak severity or frequency of the parameter. Oviduct dilatation scores also include a subjective assessment of flattening of the oviduct epithelial plicae and destruction of the oviduct mucosa.

Isolation of *Chlamydia* and measurement of chlamydial rRNA in murine cervicovaginal tissues. Cervicovaginal tissues from individual infected mice (day 4 postinfection) were processed with a Medimachine using 50- μ m Medicons (BD Biosciences) in 1 ml of 2-SP buffer. Five hundred microliters of each sample was snap-frozen for analysis of IFU, and the remaining 500 μ l was processed for RNA isolation using the Qiagen RNeasy isolation kit. The samples were analyzed for chlamydial rRNA by quantitative reverse transcription-PCR (RT-PCR), using the primers AGACAACAAGGACGCAAGAACC (forward) and GGATCATACCACCCTAACAACCTCG (reverse), as previously described (24). To isolate live elementary bodies from murine cervicovaginal tissue, 500 μ l of the lysate was sonicated and centrifuged at $100 \times g$ to remove the debris. Ten microliters was removed, diluted to 100 μ l, and overlaid on a McCoy IFU titration plate at multiple dilutions. Cells were fixed at 24 h, and IFU were enumerated as described above.

Antigen-specific T cell proliferation and serum chlamydial IgG response. Iliac nodes were processed for chlamydia-responsive T cell proliferation as described previously (5) with few modifications. Iliac nodes were excised from mice on day 7 postinfection and passed through nylon mesh to yield single-cell suspensions. Cells (2×10^5 cells/well) were seeded in a 96-well flat-bottomed tissue culture plate in complete medium (RPMI containing 10% fetal bovine serum [FBS], 2 mM glutamine, 10 mM HEPES, pH 7.4, 100 μ M nonessential amino acids, 1 mM sodium pyruvate, 50 μ M β -mercaptoethanol, and 50 μ g/ml gentamicin). *C. muridarum* grown in HeLa cells and purified on a Renografin gradient (2) was UV inactivated and used as chlamydial antigen (5 μ g/ml), in a final volume of 200 μ l. Cells treated with concanavalin A (5 μ g/ml) and cells that received no treatment were used as positive and negative controls, respectively. A parallel proliferation assay was carried out in the presence of anti-CD4 monoclonal antibody (MAb) (1 μ g/well; clone RM4-5; BD Biosciences), which sterically blocks antigen-specific T cell proliferation due to CD4-T cell receptor (TCR) coclustering (15). Cells were treated with 20 μ l of Alamar blue (Biosource) 24 h before the end of a 5-day culture, and the proliferation response was read at an excitation wavelength of 530 nm and an emission wavelength of 590 nm using a Biotek fluorescence microplate reader. A portion of cell supernatant was removed from each well immediately prior to addition of Alamar blue, and IFN- γ and IL-4 levels were measured by ELISA (R&D Systems). Chlamydia-specific Ab (IgG1 and IgG2a) responses were measured in sera collected from mice at days 21 and 42 postinfection, as previously described (5).

Flow cytometric analysis of iliac node and genital tract cells from infected mice. Cervicovaginal tissue (lower genital tract), both oviducts and the upper one-third of the uterine horns (upper genital tract), or the two oviducts alone were excised from the genital tract of an individual mouse and incubated separately with 3 ml of collagenase I (5 mg/ml) for 30 min at 37°C. Cells resuspended in 1 ml of RPMI containing 1% FBS were further treated with 0.1 ml of DNase-RNase mixture (2:18 mg/ml) to obtain a single-cell suspension. After the enzymes were neutralized with EDTA (10 μ M), cells were washed and resuspended in fluorescence-activated cell sorting (FACS) buffer (PBS, 1% bovine serum albumin [BSA], and 1 mM EDTA). Iliac nodes were also harvested, and single-cell suspensions were collected by washing the nodes through a cell strainer. Cells (2×10^5 to 5×10^5 cells/25 μ l) were incubated in Fc-Block (5 μ g/ml) for 10 min, followed by staining for individual cell surface markers or isotype controls (5 μ g/ml) for 20 min on ice. The following mAbs (BD Biosciences) were used in various combinations: CD45-peridinin chlorophyll protein (PerCP5.5) (clone 30-11), NK1.1-phycoerythrin (PE) (clone PK136), Ly6G/C-fluorescein isothiocyanate (FITC) (clone RB6-8C5) or Ly6G-FITC (clone IA8), F4/80-allophycocyanin (APC) (clone BMB), CD3-PE (clone 17A2), CD4-APC (clone RM-4), and CD8-FITC (clone 53-6.7). Neutrophils were defined as Ly6G/ C^{hi} F4/80 $^{-}$ cells, and in selected experiments an antibody specific for neutrophils (anti-1A8, which binds Ly6G only) was used for specificity. Cells were washed with FACS buffer and treated with 4',6-diamidino-2-phenylindole (DAPI) (2 μ g/ml) to stain for dead cells. Data were acquired in a FACS Aria flow cytometer (BD Biosciences) and analyzed using FlowJo software (Tree Star Inc.).

For analysis of IFN- γ production, cells harvested from cervicovaginal tissues of day 3 infected WT and MyD88 KO mice were stimulated with phorbol myristate acetate (PMA) (25-ng/ml final concentration; Sigma-Aldrich) and ionomycin (250-ng/ml final concentration; Sigma-Aldrich) for 4 h at 37°C in the presence of GolgiPlug (1:500 final dilution; BD Biosciences). Suspensions were treated as described above for surface staining using the following mAbs (BD Biosciences; same clones as above unless otherwise indicated): CD45 PE-Cy7, NK1.1-PE, and PerCP-Cy5.5 CD3e (clone 145-2C11). They were then fixed and permeabilized according to the manufacturer's instructions (BD Biosciences Cytofix/Cytoperm kit). Cells were then stained with IFN- γ -APC (clone XMGI.2) at a dilution of 1:80 for 30 min on ice. Data were acquired in a FACS Aria flow cytometer (BD Biosciences) and analyzed using FlowJo software (Tree Star Inc.).

Flow sorting of natural killer (NK) cells from cervicovaginal tissues and analysis of NK cell IFN- γ mRNA. CD45 $^{+}$ NK1.1 $^{+}$ cells were flow sorted from cervicovaginal tissues harvested from individual mice on day 4 postinfection. Cells were routinely found to be sorted to >95% purity. NK cell RNA was prepared using the RNeasy kit (Qiagen). Reverse transcription reactions and real-time RT-PCR were carried out as described previously (19). Primers for IFN- γ were generated using the Beacon Designer software (Bio-Rad) and were CCAAGCGGCTGACTGAACCT (forward) and TGGCCCGGAGTGTAGACAT (reverse). The results are presented as fold increase, by comparing the threshold cycles to the β -actin expression, using the threshold cycle ($\Delta\Delta CT$) method.

Statistics. Statistical comparisons between the WT and KO mice for levels of infection and cytokine production over the course of infection were made using a two-factor (days and murine strain) repeated-measures (RM) analysis of variance (ANOVA). A *post hoc* Tukey test was used as a multiple-comparison procedure. Kaplan-Meier survival analysis was used to compare the durations of infection in the respective strains over time. The Fisher exact test was used for determination of significant differences in frequency of hydrosalpinx between groups. The Kruskal-Wallis one-way ANOVA on ranks was used to determine significant differences in the pathological data between groups. Comparison of pathological data within groups was made using the Student-Newman-Keuls method of all-pairwise multiple comparisons. T cell proliferation data are provided as the means $\pm$ standard deviations (SD) of triplicate samples, and flow cytometric data are presented as means $\pm$ SD of percent positive cells from five tissue samples harvested on a single day; one-way ANOVA or the Student *t* test was used to determine significance between groups. Statistical tests were performed using SigmaStat software.

RESULTS

Course of *C. muridarum* infection in TLR4/MyD88 DKO mice is comparable to that in MyD88 KO mice. We and others have reported that the duration and level of primary lower genital tract infection with *C. muridarum* are significantly increased in MyD88 KO mice compared to those in WT mice (3,

19), showing that chlamydial stimulation of pathogen recognition receptors dependent on the MyD88 adaptor protein is important to host defense. To further address if the TLR4-TRIF pathway contributes to host defense against chlamydial infection in the absence of MyD88, mice deficient for MyD88 and TLR4 (TLR4/MyD88 double-knockout [DKO] mice) (24) were examined. TLR4 can signal through both MyD88 and TRIF, and generation of TLR4/MyD88 DKO mice facilitates studying the role of the TLR4-TRIF pathway in the absence of MyD88. The additional loss of signaling through the TLR4-TRIF pathway did not lead to a further increase in bacterial burden or prolongation of infection in TLR4/MyD88 DKO mice with respect to mice deficient only for the MyD88 adaptor protein (Fig. 1A and B). Likewise, mice singly deficient in TRIF (TRIF KO) displayed an infection course similar to that of WT mice (data not shown). Together these data suggest that MyD88 but not TLR4-TRIF signaling contributes to chlamydial resolution in the genital tract. Additional assessment of the hypothesis that MyD88 deficiency led to compromised early host defense against *Chlamydia* was undertaken by performance of quantitative cultures in homogenized cervical tissues of MyD88 KO and WT mice on day 4 postinfection. This method revealed that bacterial burden was significantly increased in the absence of MyD88 (Fig. 1C) on this early day of infection, supporting an important role for MyD88 signaling in the innate response to infection.

Although challenge infections are reduced in intensity and duration, sterilizing immunity does not occur in WT mice after *C. muridarum* infection (25). This relative degree of protection against a secondary infection was observed in mice deficient in MyD88 as well as those deficient in TLR4 and MyD88 (Fig. 1A). Secondary infections induced in KO mice treated with doxycycline to ensure that infection had completely resolved were abbreviated and reduced in intensity, similar to secondary infections in WT mice. The same degree of protection upon challenge was also observed in the absence of doxycycline treatment (data not shown). These data indicate that although the immune response to chlamydial infection was delayed or compromised early on, chlamydial activation of MyD88-independent pathways led to the eventual development of an effective adaptive immune response. Data from TLR4/MyD88 DKO mice suggest that the TLR4-TRIF pathway is dispensable for infection clearance in primary and challenge infection (Fig. 1A).

Natural killer cell recruitment to the cervix is normal in the absence of MyD88 signaling, but the cells' production of IFN- γ is significantly compromised. Chen et al. reported that MyD88 KO mice developed a Th2-dominant response after chlamydial genital tract infection (3). Antigen-specific CD4 T cell proliferation was not significantly different between WT, MyD88 KO, and TLR4/MyD88 DKO mice (Fig. 2A). Further, although IL-4 was observed in iliac node proliferation supernatants from MyD88 KO and TLR4/MyD88 DKO mice but not from WT mice (Fig. 2B), IFN- γ levels were significantly higher than IL-4 levels in the KO strains (4- to 5-fold) and were not different between the three groups (Fig. 2B). Therefore, we maintain that in the absence of MyD88, although the Th1 response is blunted with respect to WT mice and components of a Th2 response are observed, the overall response is predominantly Th1.

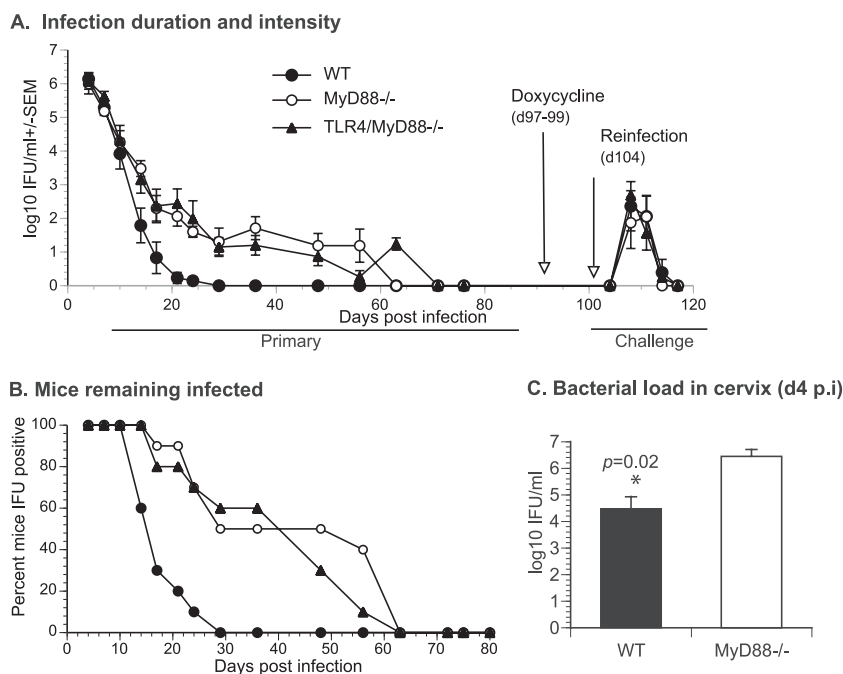


FIG. 1. The course of primary *C. muridarum* infection in TLR4/MyD88 DKO mice is comparable to that in MyD88 KO mice, and MyD88 deficiency does not alter response to challenge infection. (A) *C. muridarum* infection course in WT ($n = 10$), MyD88 KO ($n = 10$), and TLR4/MyD88 DKO ($n = 10$) mice infected with 3×10^5 IFU/mouse. Five mice were administered doxycycline and rechallenged, post-primary infection, as indicated. Log₁₀ IFU/ml were calculated from genital swabs as described in Materials and Methods and graphed as means $\pm$ standard errors of the means of animals positive for infection on that day. Significance was determined by two-way RM ANOVA (WT versus MyD88 KO mice, $P = 0.001$; WT versus TLR4/MyD88 DKO mice, $P < 0.001$; MyD88 KO versus TLR4/MyD88 DKO mice, $P > 0.05$). (B) Percentages of mice that remain infected in each group are indicated; P is 0.001 by Kaplan-Meier survival analysis for WT versus MyD88 KO and TLR4/MyD88 DKO mice. Five mice per group were analyzed individually in a single experiment, and the data represent the combined results of two individual experiments. (C) Chlamydial IFU in cervical tissues from WT ($n = 5$) and MyD88 KO ($n = 5$) mice obtained at day 4 postinfection. The asterisk denotes a statistically significant difference by Student's t test. One independent experiment was conducted with five mice analyzed per group.

To investigate a potential mechanism for the blunted Th1 response observed in the KO strains, we examined NK cell recruitment and these cells' IFN- γ production in the cervical tissues of infected mice. Natural killer cells are major producers of IFN- γ early in chlamydial genital infection and are necessary for generation of an optimal Th1 response (28). Significantly lower IFN- γ levels were observed in the genital secretions of MyD88 KO and TLR4/MyD88 DKO mice than in those of WT mice on days 2 to 5 postinfection (Fig. 3A). We hypothesized that either influx of NK cells and/or production of NK cell-derived IFN- γ was compromised in the absence of MyD88. Flow cytometric analysis of the percentages of NK1.1⁺ cells in cervical tissues of WT, MyD88 KO, and TLR4/MyD88 DKO mice on day 4 postinfection revealed no differences between the groups (Fig. 3B). However, when equal numbers of NK1.1⁺ cells were FACS sorted (>95% purity) and processed for IFN- γ protein by ELISA, there was no detectable level of IFN- γ protein in MyD88 KO NK1.1 cells, compared to WT cells (Fig. 3C). Furthermore, measurement of IFN- γ mRNA in an independent sorting of cervical NK1.1⁺ cells of KO and WT mice on day 4 postinfection revealed that this defect in MyD88KO NK1.1 cell IFN- γ production occurred at the level of transcription (Fig. 3D). The IFN- γ mRNA data were also normalized to NK1.1 expression to confirm the source of IFN- γ , and similar results were obtained (data not shown). To confirm that our sorting data reflected

differences in NK cells and not natural killer T (NKT) cells, we analyzed cells harvested from cervicovaginal tissues of WT and MyD88 KO mice 3 days postinfection using intracellular cytokine staining and flow cytometry. More than 90 to 95% of NK1.1 cells were negative for CD3, suggesting that NK and not NKT cells predominate in the cervix during these early days of infection. The frequencies of IFN- γ -producing NKT cells (NK1.1⁺ CD3⁺) were not different between the groups: WT, 33.4% $\pm$ 14.1%, and KO, 28.2% $\pm$ 11.0%. However, the frequency of IFN- γ -producing NK cells (NK1.1⁺ CD3⁻) cells was significantly higher in WT mice than in MyD88 KO mice: WT, 4.0 $\pm$ 2.4, and KO, 27.3 $\pm$ 8.3 ($P = 0.002$) (Fig. 3E). Thus, it appears that MyD88 signaling is required for induction of this important Th1 cytokine in NK cells infiltrating the cervix in response to chlamydial infection. TLR4/MyD88 DKO mice displayed a phenotype with respect to IFN- γ protein and mRNA similar to that of the MyD88 KO mice (data not shown).

Decreased IFN- γ in cervical tissues is associated with reduced levels of IL-17 and IL-18 but not IL12p70. To address the possible mechanism whereby MyD88 deficiency leads to NK cell dysfunction during chlamydial genital infection, cervical homogenates were examined for IL-12p70, IL-18, and IL-17, cytokines classically associated with NK cell activation. A significant reduction in the level of IFN- γ protein was detected in cervical homogenates of MyD88 KO mice on day 3 com-

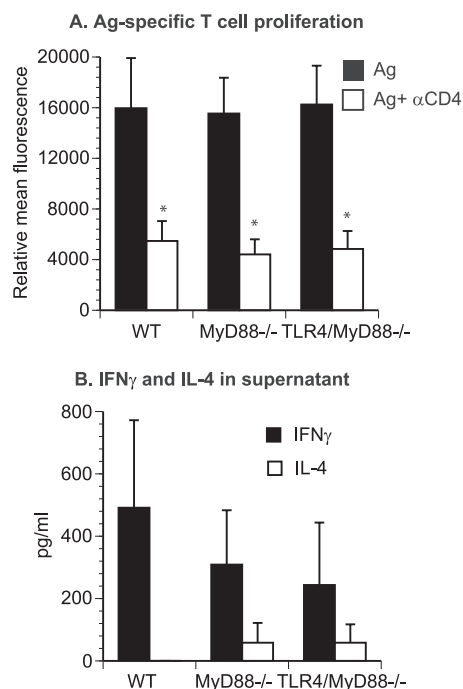


FIG. 2. Chlamydia-specific T cell proliferation is not altered by MyD88 deficiency, but a slight reduction in IFN- γ production and detection of IL-4 indicate inefficient suppression of a Th2 response. (A) Iliac lymph nodes obtained from infected mice at day 7 postinfection ($n = 3$ /group) were subjected to T cell proliferation assay as described in Materials and Methods. Anti-CD4 MAb was used to block antigen (Ag)-specific proliferation. Proliferation was measured using Alamar blue fluorescence. Data are represented as means $\pm$ SD of values obtained from individual animals after subtraction of medium control wells, from a single experiment, representative of two experiments; *, $P < 0.01$ for untreated versus CD4 Ab-treated groups. (B) Means $\pm$ SD of IFN- γ and IL-4 levels in supernatants of proliferation assay measured by ELISA; P is < 0.05 for IL-4 for individual KO strains compared to WT.

pared to WT mice (Fig. 4A) despite detection of similar levels of IL-12p70 (Fig. 4B). IL-18 levels were partially reduced (50% decrease) in the absence of MyD88 (Fig. 4C), and IL-17, a cytokine recently described as a key inducer of NK cell IFN- γ production in the lung (17), was barely detectable (Fig. 4D). Similar results were also obtained from TLR4/MyD88 DKO mice tested at day 2 postinfection (data not shown). Thus, MyD88-independent induction of IL-12p70 is not sufficient to drive NK cell production of IFN- γ in the absence of IL-17 and lowered levels of IL-18.

Macrophage and neutrophil influx into genital tract tissues is not compromised in the absence of MyD88 and TLR4/MyD88, but CD4⁺ T cell numbers are reduced. Despite a reduced Th1 response, the MyD88 KO and TLR4/MyD88 DKO mice eventually clear infection, suggesting that alternative mechanisms aid in bacterial clearance. To determine these mechanisms, genital tracts were examined for the recruitment of inflammatory cells. During the early days of infection, no decrease was observed in the percentages of CD45⁺ inflammatory cells recruited to the cervix and upper genital tract tissues (horns and oviducts) of these mice compared to WT mice (Fig. 5A and B). The percentages of Ly6G^{hi} F4/80⁻

neutrophils found in the cervical tissues on days 4 and 14 postinfection (Fig. 5C) and in the upper genital tract tissues on days 7 and 14 postinfection (Fig. 5D) were not different in the KO and WT mice, indicating that recruitment of neutrophils was not compromised by the absence of MyD88. Neutrophils were actually increased in the cervical tissues of the KO strains on day 7 postinfection, with the increase being statistically significant in the MyD88 KO mice compared to WT mice on this day (Fig. 5C). In the cervix, the percentages of F4/80⁺ macrophages were not significantly different between the groups on any day examined (Fig. 5E). Indeed, macrophages were significantly increased in the upper genital tract tissues of MyD88 KO and TLR4/MyD88 DKO mice compared to WT mice on days 7 and 14 (Fig. 5F). The detection of increased numbers of neutrophils and macrophages in the genital tract tissues of the KO strains likely reflects the increased bacterial burden observed in these strains compared to WT (Fig. 1C). Detection of similar percentages of CD4⁺ T cells in comparison to WT on day 7 in both the lower and upper genital tract tissues indicated that early recruitment of CD4⁺ T cells in the KO strains was normal (Fig. 5G and H). However, on day 14 postinfection, CD4⁺ T cells were significantly reduced in the lower and upper genital tract tissues of the KO strains compared to WT (Fig. 5G and H). This may reflect decreased ongoing recruitment as a consequence of decreased chemokine expression, decreased proliferation of CD4⁺ T cells, or decreased longevity of these cells at the local site in the absence of MyD88 signaling.

MyD88-dependent and -independent cytokine and chemokine responses are observed in the genital tract after infection.

To understand the mechanism of cellular recruitment in the absence of MyD88, we examined cytokine and chemokine levels in genital secretions. As shown previously in MyD88 KO mice (3), markedly decreased levels of TNF- α (Fig. 6A) and IL-6 (data not shown) were observed in the genital tract secretions of MyD88 KO and TLR4/MyD88 DKO mice through day 10 of infection. These data indicate either that recruitment of inflammatory cells is independent of these cytokines or that redundant mechanisms exist for eliciting cellular recruitment. In fact, a number of cytokines/chemokines were determined to be only partly dependent on or independent of MyD88 signaling. For instance, among the chemokines, although the neutrophil chemokine CXCL1 (KC) was significantly reduced throughout the first week of infection (Fig. 6C), the levels of monocyte-inducing chemokine CCL2 (monocyte chemoattractant protein 1 [MCP-1]) were similar (days 2 to 4) or significantly higher (days 5 to 9) in the secretions of MyD88 KO and TLR4/MyD88 DKO mice than in those of WT mice (Fig. 6D). The T cell chemokines CXCL9 (MIG) (Fig. 6E) and CXCL10 (IP-10) (19; also data not shown) were significantly reduced in the genital tract secretions of the KO strains compared to WT, though both of these chemokines were detected at low to moderate levels in the absence of MyD88. The levels of T cell chemokine CCL5 (RANTES) were found not to be significantly different between the three groups (Fig. 6F). These results suggest that CXCL1-independent neutrophil recruitment occurs in the absence of MyD88 and implicate CCL2 in the enhanced recruitment of monocytes/macrophages in the KO strains. In addition, although CCL5 is not sufficient for normal T cell recruitment, its continued induction in the ab-

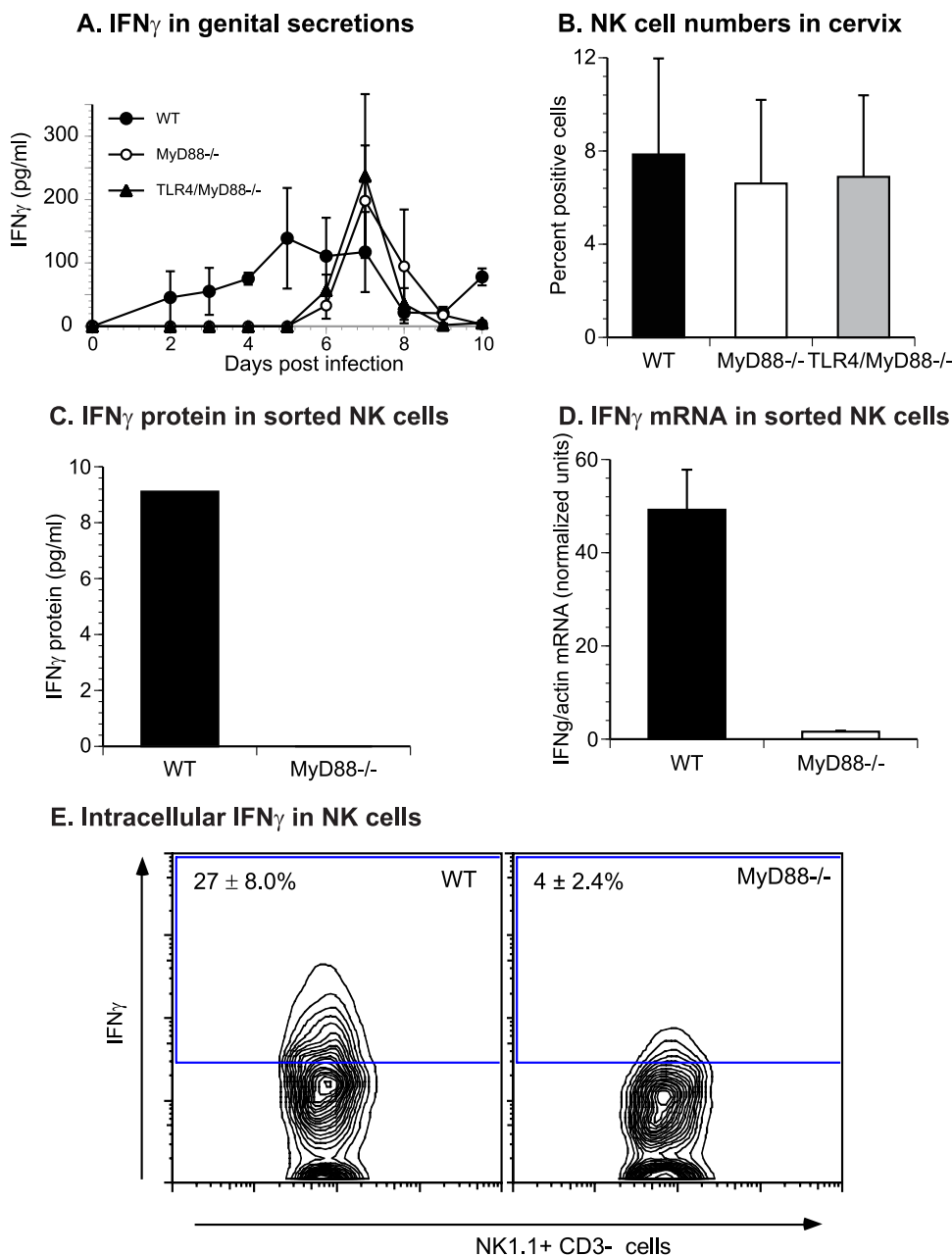


FIG. 3. Reduced IFN- γ in genital secretions early in infection is due to inability of NK1.1 cells to produce IFN- γ . (A) Genital secretions obtained at different time points postinfection were analyzed for IFN- γ by ELISA. Five mice per group were analyzed in a single experiment, and the data are representative of two individual experiments. (B) Flow cytometric analysis of NK cells in the cervix at day 4 postinfection ($n = 4$ /group). Cells were gated for live CD45 $^{+}$ cells and analyzed for NK1.1 expression. Data are percentages of gated cells from total live cell populations and are from one independent experiment. (C) IFN- γ protein levels from 30,000 NK1.1 cells determined by ELISA. (D) IFN- γ mRNA transcript levels normalized to actin levels in NK1.1 cells sorted from the cervix of WT and MyD88 KO mice ($n = 5$; pooled samples from one independent experiment) at day 4 postinfection. (E) Intracellular staining of IFN- γ in NK1.1 $^{+}$ CD3 $^{-}$ cells from cervical tissues from WT ($n = 5$) and MyD88 KO ($n = 5$) mice obtained at day 3 postinfection in one independent experiment; P is 0.003 by Student's t test.

sence of MyD88, together with partial induction of additional T-cell chemokines, likely results in the eventual recruitment of this important cellular arm of chlamydial host defense.

Interestingly, although IL-1 β levels (Fig. 6B) were extremely low in secretions of MyD88 KO and TLR4/MyD88 DKO mice for the first 4 to 5 days of infection, levels of this cytokine increased to those observed in WT mice by day 6 to 7. The

delayed detection of IL-1 β and IFN- γ (Fig. 3A) suggests that cells recruited to the cervix by day 6 were able to secrete these mediators via signaling pathways independent of MyD88. The recovered IL-1 β response likely results from the influx of macrophages and polymorphonuclear leukocytes (PMNs) into the cervix, which we showed recently to be the predominant cell sources for IL-1 β during *C. muridarum* genital tract infection

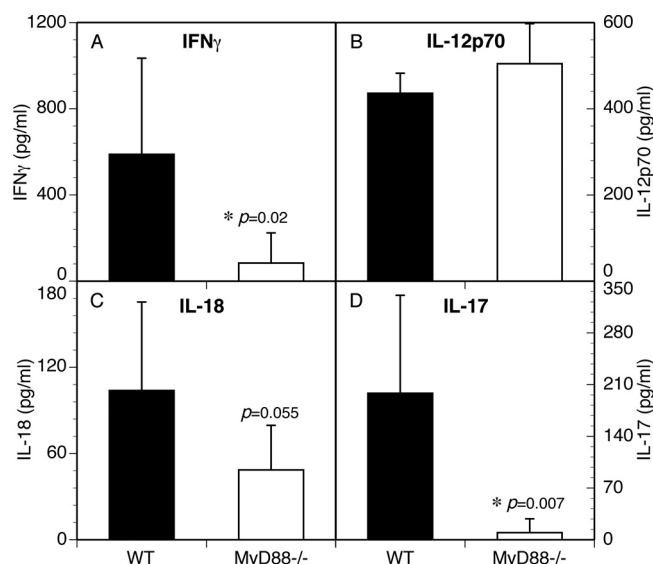


FIG. 4. MyD88 deficiency leads to decreases in tissue levels of NK cell agonists IL-18 and IL-17 but normal levels of IL-12p70 early during infection. Cervical homogenates from WT and MyD88 KO mice ($n = 5$ /group) were analyzed for IFN- γ (A), IL-12p70 (B), IL-18 (C), and IL-17 (D) by ELISA. P values were determined by Student's t test, and data represent means $\pm$ SD of values obtained from 5 mice per group in a single independent experiment.

(24), while the source for IFN- γ at these time points is likely T cells, based on the T cell proliferation data (Fig. 2B). All of the cytokines and chemokines analyzed were detected at similar levels in MyD88 KO and TLR4/MyD88 DKO mice, indicating no additional role for the TLR4-TRIF pathway in their production.

Chronic oviduct damage in MyD88 KO and TLR4/MyD88 DKO mice is comparable to that observed in WT mice. Previously, we observed less oviduct pathology in TLR2 KO mice, which display a course of infection similar to that of WT mice (8). The reduced pathology was attributed to lower production of proinflammatory cytokines, including TNF- α . Despite reduced proinflammatory cytokines early on during infection in MyD88 KO and TLR4/MyD88 DKO mice, the frequency of oviduct hydrosalpinx observed 42 and 56 days post-primary infection in the KO strains was not significantly different from that in WT mice. In fact, histological examination of oviducts showed that the KO strains exhibited dilation scores that were similar to those of WT mice after both primary (Fig. 7A) and challenge (data not shown) infection. Furthermore, similar levels of neutrophils, lymphocytes, and monocytes and plasma cell infiltrates were observed in the oviducts of all three groups at these late time points (Fig. 7B).

To determine the mechanism for development of oviduct damage in the absence of MyD88 signaling, additional groups of mice were sacrificed earlier (day 14) during infection and the cellular and cytokine responses were examined in oviduct homogenates. This time point was chosen since we have determined that bacterial burden is decreasing but inflammatory cells are still prominent in the oviducts on this day in WT C57BL/6 mice (data not shown). We hypothesized that MyD88-independent responses in the oviducts of the KO

strains could lead to development of oviduct tissue damage associated with a more chronic infection. As observed by Chen et al. (3), increased numbers of bacteria (100-fold more) were observed in oviducts of mice deficient for MyD88 compared to WT mice (Fig. 7C). The presence of increased amounts of bacteria led to an inflammatory cytokine response independent of MyD88, since comparable levels of IL-1 β were detected in the oviducts of all three strains (data not shown). Further, the MyD88-independent CCL2 response was significantly elevated in oviducts of the MyD88 KO and TLR4/MyD88 DKO mice (Fig. 7D) compared to WT mice. It is likely that the enhanced production of CCL2 promoted enhanced monocyte/macrophage influx into the upper genital tract, including the oviducts, and that these cells secreted the proinflammatory molecule IL-1 β independently of MyD88, leading to oviduct tissue damage.

Increased granulomatous inflammation is observed in the uterine horns of MyD88 KO and TLR4/MyD88 DKO mice. In contrast to the oviducts, examination of the uterine horns of mice sacrificed on day 42 postinfection revealed significant increases in lymphocytic/monocytic and plasma cell infiltrates in both MyD88 KO and TLR4/MyD88 DKO mice compared to WT mice (Fig. 8A). These data are consistent with flow cytometric data on day 14 postinfection, where more F4/80⁺ macrophages were observed in the KO strains (Fig. 5F), and with the detection of increased levels of MCP-1 (CCL2) in genital tract secretions (Fig. 6D). Additionally, granulomatous inflammation and organized granulomas were readily observed in the uterine horns of the KO strains on day 42 (Fig. 8B and C), with greater numbers of granulomas being found in the TLR4/MyD88 DKO mice (median granuloma score, 4 for TLR4/MyD88 DKO versus 2 for MyD88 KO; $P < 0.05$ by ANOVA on ranks). In the uterine horns of the WT mice, organized granulomas were rarely observed (data not shown). By day 56 postinfection, although organized granulomas were decreased in the horns of the KO strains, residual histiocytic inflammation persisted. This was in contrast to the WT mice, where inflammation had completely resolved (data not shown). Interestingly, the granulomas observed in the KO strains randomly showed positive staining for chlamydiae (Fig. 8D). These data suggest that monocyte/macrophage influx and the development of organized granuloma may be a compensatory response that develops in the absence of MyD88 in an attempt to contain and resolve infection, an event that is more pronounced in the absence of both TLR4 and MyD88.

DISCUSSION

In this study we analyzed the mechanisms involved in delayed bacterial clearance during *C. muridarum* genital infection in mice deficient for the central adaptor molecule MyD88. It has been previously shown that MyD88 KO mice display a blunted Th1 response and Th2 polarization (3). In this study we maintain that the adaptive CD4 T cell response generated in the absence of MyD88 signaling remains Th1 in character, although blunted in degree. We show that NK cell production of IFN- γ is compromised in MyD88 KO mice and that this likely leads to the observed reduction in the Th1 response. Multiple immune responses were compromised in the absence of MyD88 signaling, including the early inflammatory cytokine

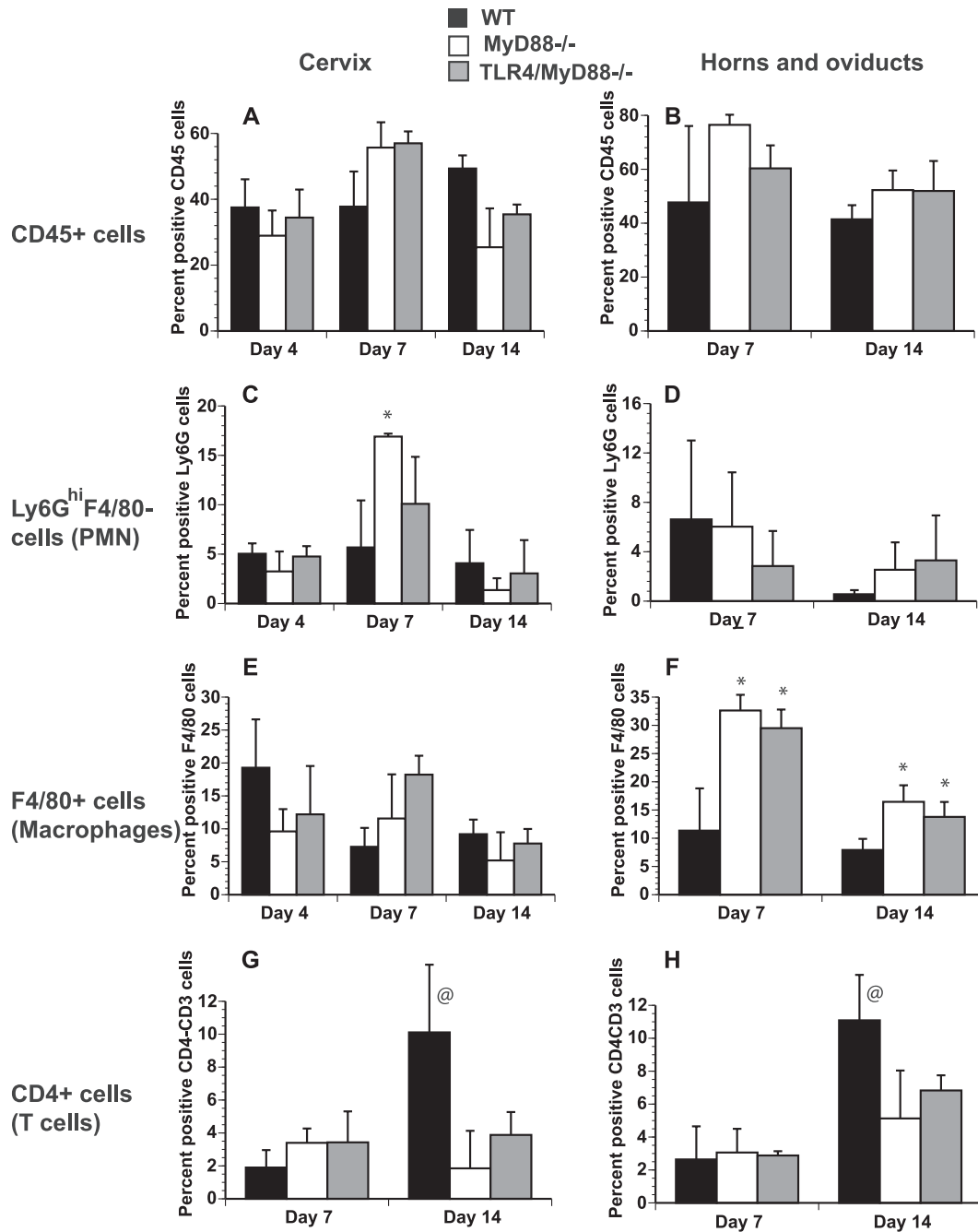


FIG. 5. Recruitment of innate immune cells is not compromised in the absence of MyD88, but CD4 T cells are reduced during peak levels of infection. Flow cytometric analysis of leukocytes in the cervix (A) and upper genital tract (B), at days 4, 7, and 14 postinfection in WT, MyD88 KO, and TLR4/MyD88 DKO mice ($n = 3$ mice/group). Cells were gated for live CD45⁺ cells and analyzed for Ly6G^{hi} F4/80⁻ (C and D), F4/80⁺ (E and F), or CD4⁺ (G and H) expression. Data are percentages of gated cells from total cell populations. (C) *, $P < 0.05$ for MyD88 KO versus WT mice; (F) *, $P < 0.05$ for both KO strains versus WT; (G and H) @, $P < 0.05$ for WT versus both KO strains by one-way ANOVA. Data represent means $\pm$ SD of percentages obtained from 3 mice per group in an individual experiment that is representative of two experiments performed independently.

response and genital tract CD4 T cell infiltration. Importantly, although MyD88 played a role in optimizing Th1 polarization, it was not necessary for Th1 priming since normal levels of IL-12p70 were detected in its absence. It is possible that this intact, but blunted, Th1 response contributes to eventual eradication of infection, although the detection of a heightened

macrophage response may represent a compensatory host defense mechanism. Furthermore, no additional protection is offered through TLR4-TRIF signaling, as TLR4-MyD88 DKO mice display an infection clearance course similar to that of MyD88 KO mice.

The requirement for MyD88 appears to vary with respect to

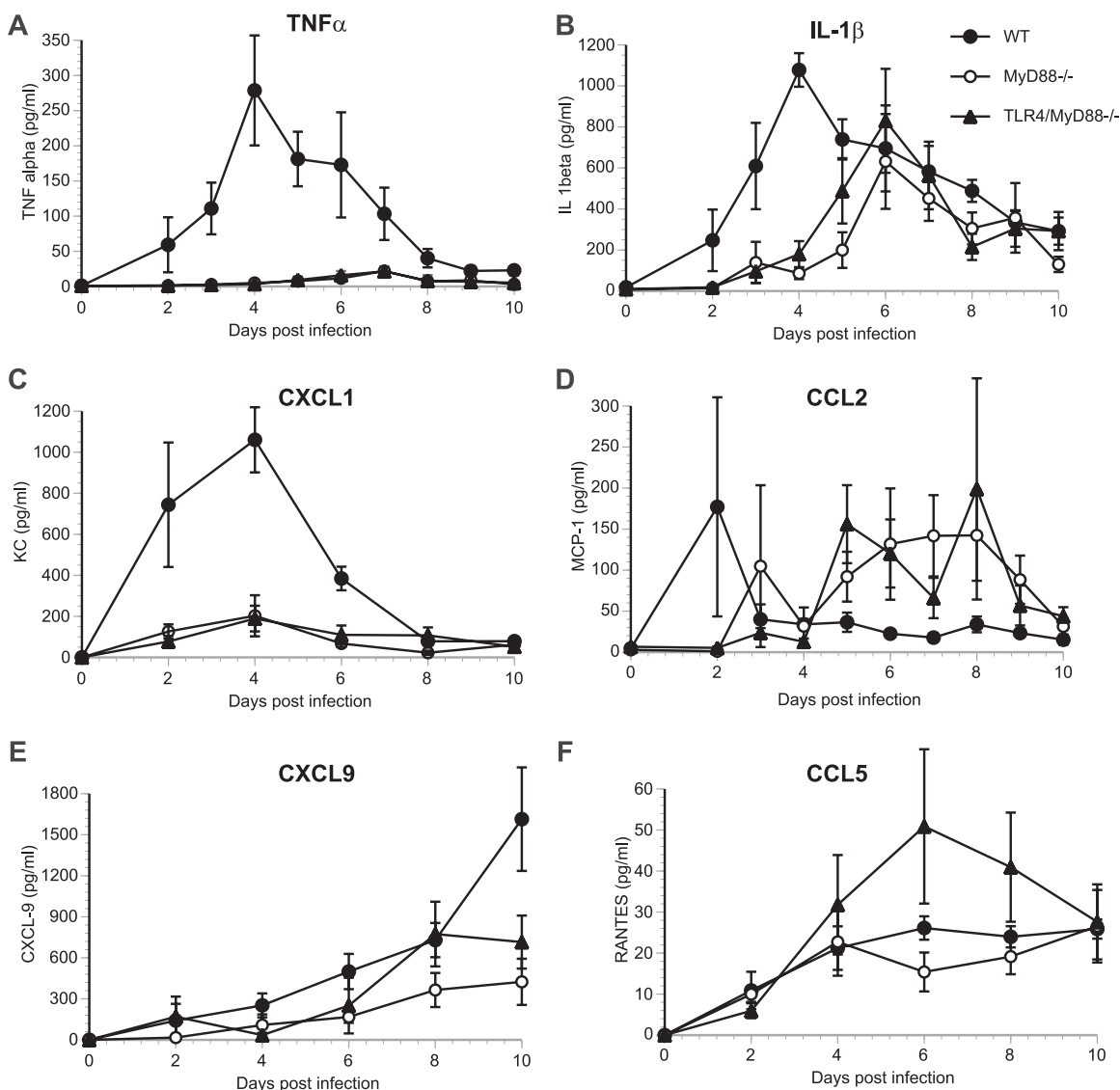


FIG. 6. Differential effect of MyD88 deficiency on cytokine and chemokine responses detected in genital tract secretions. Genital secretions obtained at different time points postinfection were analyzed for TNF- α (A), IL-1 β (B), KC (CXCL1) (C), MCP-1 (CCL2) (D), MIG (CXCL9) (E), and RANTES (CCL5) (F) using a Milliplex cytokine array. Two-way RM ANOVA revealed a P value of <0.05 for TNF- α , KC, and IL-1 β for comparison of both KO strains with WT. For MCP-1 (CCL2), P was 0.015 for WT versus both KO strains on days 5 to 10. Data represent means $\pm$ SD of values obtained from 5 mice per group in an individual experiment that is representative of two experiments performed independently.

chlamydial strain and the site of infection. In a lung infection model with *Chlamydia pneumoniae*, MyD88-deficient mice were unable to upregulate proinflammatory cytokines or recruit neutrophils to the lung, and recruitment of CD8⁺ and CD4⁺ T cells was delayed. MyD88 KO mice were unable to clear the pathogen from their lungs and exhibited greater mortality than did WT mice (20). Similarly enhanced bacterial burden and mortality were recently reported after airway infection of MyD88 KO mice with *C. muridarum*, where lack of early IL-17 production was found to be a key mechanism responsible for enhanced susceptibility (31). A recent study also showed a delayed clearance of genital infection in MyD88 KO mice and a shift toward a Th2 response (3). A key finding of our study that is distinct from the lung infection model is the

observation of normal recruitment of innate immune cells to the genital tract in the absence of MyD88 at day 4 postinfection. It is quite possible that MyD88 could have an effect on cellular recruitment immediately after infection. However, tissue-specific MyD88-independent responses are rapidly operational in infected genital tract epithelial cells. Despite normal recruitment of innate immune cells to the genital tract early in infection, reduced levels of inflammatory cytokines and IFN- γ were observed, indicating that the cells entering the infected site are less efficient in making these cytokines. Specifically, NK cells in MyD88-deficient mice were found to have reduced levels of IFN- γ transcripts and intracellular IFN- γ protein. Previously, depletion of NK cells using anti-asialo-GM-1 antibody during chlamydial genital infection led to a similar phe-

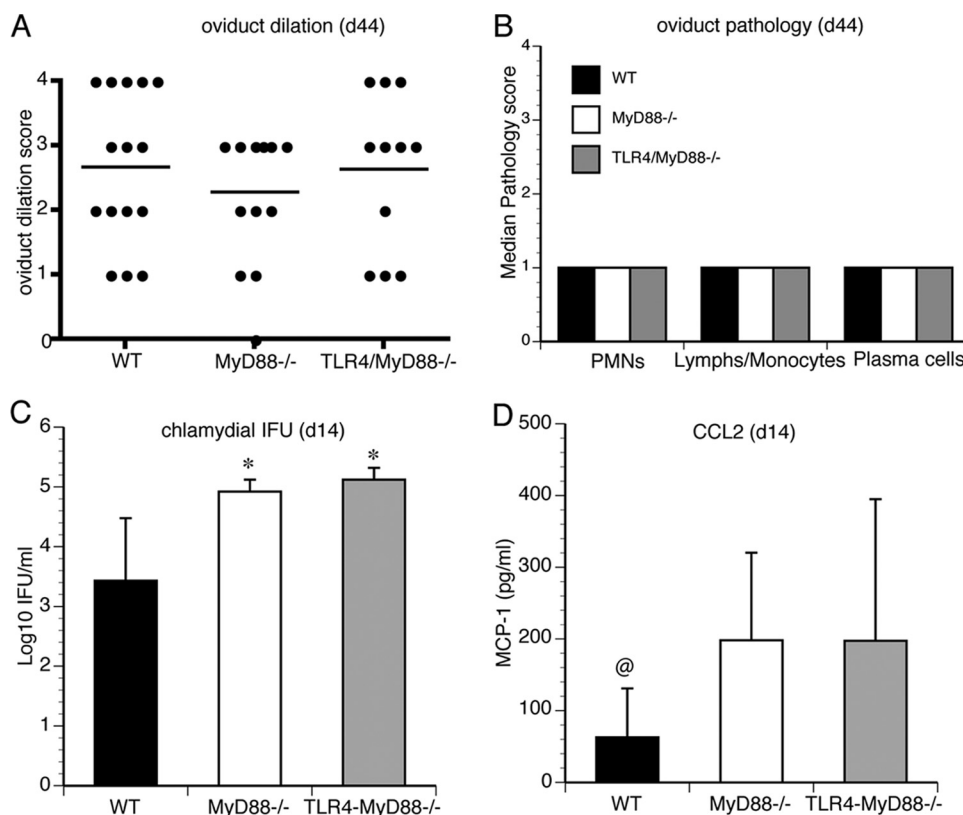


FIG. 7. Oviduct histopathology is not different in WT, MyD88 KO, and TLR4/MyD88 DKO mice. (A) An oviduct dilation score was assigned to each mouse individually based on the pathological exam of both oviducts at day 44 or 56 postinfection by a pathologist blinded to the experimental design. Data were obtained from WT ($n = 15$), MyD88 KO ($n = 14$), and TLR4/MyD88 DKO ($n = 11$) mice. Data were compiled from two independent experiments. (B) Median pathological scores for acute infection (PMNs), chronic infection (lymphocytes/monocytes), and plasma cells in histopathological sections of oviducts at day 44 postinfection from a single independent experiment with WT ($n = 7$), MyD88 KO ($n = 7$), and TLR4/MyD88 DKO ($n = 5$) mice. (C and D) Oviducts from infected WT ($n = 8$), MyD88 KO ($n = 5$), and TLR4/MyD88 DKO ($n = 4$) mice were harvested at day 14 postinfection and analyzed for chlamydial IFU (C) and for MCP-1 (CCL2) by Milliplex assay (D). Data are means $\pm$ SD of values obtained from each group of mice in a single independent experiment. Significance was measured by one-way ANOVA. *, $P < 0.05$ for WT versus both KO strains; @, $P < 0.05$ for WT versus MyD88 KO mice.

notype of delayed clearance and a shift away from Th1 and toward Th2 (28). This would suggest that the NK cell IFN- γ defect is likely responsible for ineffective suppression of the Th2 response and decreased intensity of the Th1 response, contributing to delayed clearance of infection in MyD88 KO mice.

Investigation of the mechanism for MyD88-dependent NK cell dysfunction revealed that levels of the NK cell agonists IL-18 (9) and IL-17 (17) were decreased in cervical homogenates. In addition, both of these cytokines along with TNF- α were absent from genital tract secretions of MyD88-deficient mice, suggesting that their induction is largely MyD88 dependent. Since IL-18, IL-17, and TNF- α (30) are all important activators of NK cells, it is possible that the combined lack of TNF- α and IL-17 and reduced IL-18 are responsible for the lack of NK cell IFN- γ production. A recent report revealed a key individual role for IL-17 in the induction of lung NK cell IFN- γ production during *Francisella tularensis* infection (17). However, the individual roles for IL-18, IL-17, and TNF- α in NK cell IFN- γ production in the genital tract need further investigation, and it is possible that MyD88 deficiency alters class I expression during chlamydial infection, leading to de-

creased NK cell activation. Further, it is not clear what drives the induction of IL-12p70 in the absence of MyD88. Since TLR4/MyD88 DKO mice also displayed normal IL-12p70 levels (data not shown), one can surmise that chlamydia-induced IL-12p70 production is controlled by TLR-independent pathways.

We observed that chlamydia-specific iliac node CD4 T cell proliferation at day 7 postinfection was unaffected by the absence of MyD88 signaling, indicating no inherent proliferative defect in MyD88 KO T cells. Additionally, the levels of IFN- γ in genital secretions were found to be restored to normal WT levels by day 7 postinfection. Thus, unlike the NK cells, the CD4 T cells did not display a defect in IFN- γ production but responded to the normal levels of IL-12p70 induced in the absence of MyD88. This demonstrated a differential requirement for MyD88 by NK cells and CD4 T cells for IFN- γ production. However, despite normal T cell proliferation in the draining nodes, reduced numbers of CD4 T cells were observed at the peak of infection in the genital tract. Possible reasons for this phenotype could include reduced induction of MIG (CXCL9) and IP-10 (CXCL10). The reduced recruitment of T cells is likely an important contributing factor to the

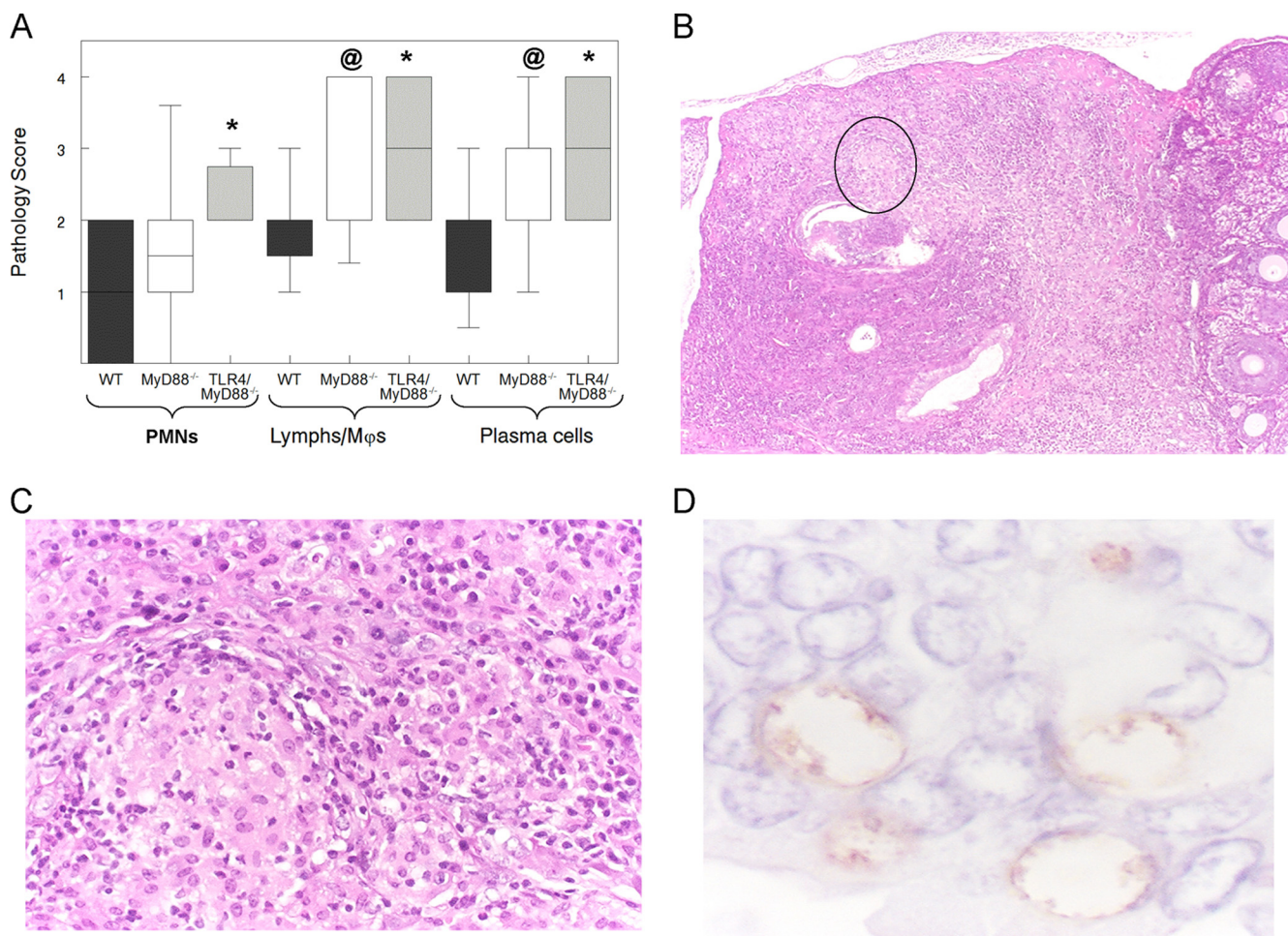


FIG. 8. Uterine horn tissues from both MyD88 KO and TLR4/MyD88 DKO mice contained enhanced degrees of mononuclear cell infiltrates associated with granulomatous inflammation and detection of chlamydial antigens in areas of granulomas. (A) Lymphocytes, monocytes/macrophages, and plasma cell infiltrates were increased in the uterine horns of both KO strains compared to WT in tissues harvested 44 days postinfection. Boxes extend from the 25th to 75th percentiles, and bars extend from the 5th to 95th percentiles; data are from the combined analysis of several mice per group in a single independent experiment with WT ($n = 7$), MyD88 KO ($n = 7$), and TLR4/MyD88 DKO ($n = 5$) mice. *, $P < 0.05$ for TLR4/MyD88 DKO versus WT mice; @, $P < 0.05$ for MyD88 KO versus WT mice. (B) Well-formed granulomas were observed in the uterine horns of both of the KO strains but were not observed in WT mice. Representative section from uterine horn of TLR4/MyD88 DKO mouse (magnification, $\times 10$). (C) Higher magnification ($\times 40$) of granuloma circled in panel B. (D) Immunohistochemical staining of tissue at site of granuloma reveals positive staining for chlamydial antigen ($\times 100$).

delayed clearance of infection in MyD88-deficient mice. However, at this point one cannot rule out that expression of MyD88 in CD4 T cells might be necessary for efficient clearance of infection, as observed during *Toxoplasma gondii* infection (16).

Considering the ability of chlamydiae to interact with multiple innate immune receptors that can play redundant roles in propagation of immune responses, compensatory mechanisms are likely activated in the absence of MyD88 signaling. The monocyte recruiting chemokine MCP-1 (CCL2) was elevated in MyD88 KO and TLR4/MyD88 DKO genital tract secretions. Type I IFNs may be involved in the induction of MCP-1 in this infection model, since signaling through the type I interferon receptor (IFNAR) has been implicated in MCP-1 induction (12) and we have found that type I IFNs can be produced independently of MyD88 during chlamydial infection (23). The elevated MCP-1 levels represent a potential

mechanism for increased recruitment of monocytes/macrophages in the KO strains. Early in infection, increased numbers of macrophages were detected in the upper genital tract tissues of the KO strains by flow cytometric analysis (Fig. 5F). Histologic examination of genital tract tissues on day 42 postinfection revealed the development of organized granulomas in the uterine horns of MyD88-deficient mice. It is possible that during chronic infection in the absence of MyD88, granulomas develop in an attempt to control and localize infection, and this is reminiscent of the host response to chronic infection with *Mycobacterium tuberculosis*. Interestingly, granulomas were increased in TLR4/MyD88 DKO mice. This may be explained by evidence that in the absence of TLR4-induced signaling through Toll/IL-1R domain-containing adaptor inducing IFN- β (TRIF), macrophages are resistant to apoptosis, as observed during *Yersinia* infection (26). However, it is not clear how increased macrophage recruitment is beneficial for infec-

tion clearance, since *C. muridarum* grows in macrophages and could infect and grow in MyD88-deficient macrophages, as evidenced by past *in vitro* data (19) and present immunohistochemical analysis (Fig. 8D). Another potential role for the observed increase in macrophages in the KO mice could be related to their scavenger function. Their recruitment would allow for engulfment of dead neutrophils and sloughed epithelial cells damaged from prolonged infection.

Overall, the early inflammatory response observed in the absence of MyD88 indicates the dichotomy between normal recruitment of inflammatory cells and the inability of these cells to produce sufficient cytokines to effectively control infection early on. As a result, the early bacterial burden in the cervix of MyD88 KO mice is 100-fold greater than that in WT mice. The blunted Th1 response and decreased T cell infiltration of the genital tract by T cells likely lead to continued lack of control of bacterial replication. Consequently, an elevated level of ongoing infection in the oviducts along with compensatory MCP-1/CCL2 and macrophage responses promotes continued production of molecules such as IL-1 β , which is MyD88 independent by day 7 postinfection. The prolonged infection thereby promotes tissue destruction leading to chronic oviduct pathology. *In vitro* data from Hvid et al. (11) showing that chlamydia-induced damage to human Fallopian tube cells is directly related to production of IL-1 and detection of decreased oviduct pathology in IL-1 β KO mice (24) further support this possibility. In these respects the MyD88 KO mice differ from the TLR2 KO mice, where lower levels of inflammatory cytokines were associated with a significant decrease in oviduct pathology, since infection clearance was normal. Additionally, chronic infection promotes enhanced plasma cell responses and heightened serum antibody levels. These combined responses lead to eventual resolution of infection and resistance to challenge infection equal to those of WT mice.

Although we have defined several mechanisms responsible for the infection and pathology phenotypes observed in MyD88-deficient mice, several questions remain unanswered. The first relates to the innate immune receptor pathway that is the driving force for induction of the important Th1-inducing cytokine, IL-12p70, which results in priming of T cells during chlamydial infection being largely MyD88 independent. Second, the trigger for the compensatory enhanced MCP-1/CCL2 response remains undefined. The importance of the signaling pathways that operate in the absence of MyD88 to ultimately clear chlamydial infection cannot be understated, as these contribute to the remarkable flexibility of the immune system, ensuring generation of an adaptive response despite loss of an important arm of innate immunity.

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