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Antibody, but not B cell-dependent antigen presentation, plays an essential role in preventing *Chlamydia* systemic dissemination in mice

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Abstract

The obligate intracellular bacterium *Chlamydia trachomatis* causes the most prevalent bacterial sexual transmitted infection worldwide. CD4 T cells play a central role in protective immunity against *Chlamydia* female reproductive tract (FRT) infection, while B cells are thought to be dispensable for resolution of primary *Chlamydia* infection in mouse models. We recently reported an unexpected requirement of B cells in local *Chlamydia*-specific CD4 T cell priming and bacterial containment within the FRT. Here, we sought to tackle the precise effector function of B cells during *Chlamydia* primary infection. Using mixed bone marrow chimeras that lack B cell-dependent Ag presentation ($MHCII^{B-/-}$) or devoid of circulating antibodies ($AID^{-/-}$ x $\mu S^{-/-}$), we show that *Chlamydia*-specific CD4 T cell expansion does not rely on Ag presentation by B cells. Importantly, we demonstrate that antibody, but not B cell-dependent antigen presentation, is required for preventing systemic bacterial dissemination following *Chlamydia* FRT infection.

Keywords

Antibody; antigen presentation; B cells; infection; Chlamydia

Introduction

Chlamydia is a gram-negative obligate intracellular bacterium that causes diseases at various mucosal tissues. *Chlamydia trachomatis* ocular infection leads to inclusion conjunctivitis and trachoma, the leading cause of infectious blindness worldwide[1]. Sexually transmitted *C. trachomatis* are tropic for the transitional and columnar epithelial cells in the female reproductive tract (FRT)[2]. While most *Chlamydia* sexually transmitted infections (STIs) are asymptomatic, pathological immune responses can often times lead to severe tissue damage and result in pelvic inflammatory disease, ectopic pregnancy and infertility [3]–[7].

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Conflict of interest

The authors declare no financial or commercial conflict of interest.

In addition to the most prevalent STI *C. trachomatis* strains (serovars D through K), the invasive lymphoma granuloma venereum (LGV) strains (serovars L1-L3) are associated with several bacterial STI outbreaks in recent years[8]–[10]. These strains evade the local draining lymph nodes and disseminate to surrounding tissues[11]. Despite relatively rare clinical presentations, increasing evidence suggests that *C. trachomatis* infection causes diseases at remote tissues away from the initial mucosal portal of entry, such as arthritis in joints, Fitz-Huge-Curtis syndrome in the peritoneal cavity and chronic colonization in the gastroenteric tract[12]–[17]. Another species, *Chlamydia pneumoniae*, the etiologic agent for community-acquired pneumonia, is associated with exacerbation of cardiovascular diseases in both mouse models and clinical studies[18]–[20]. These findings suggest that despite mucosal epithelium tropism, *Chlamydia* infection in otherwise healthy individuals is likely under strict immune surveillance to prevent systemic spread. However, the host immune components that keep *Chlamydia* contained at mucosal tissues in immunocompetent hosts remain largely undefined.

Chlamydia muridarum infection in female mice closely resembles many features of *C. trachomatis* infection in women, including protective immune responses, development of immunopathology[21]–[23]. Using the mouse model of *C. muridarum* infection, research in the past a few decades has established a central role of cell-mediated immunity (CMI) against *Chlamydia*[24],[25]. In contrast, B cells or antibody-mediated immunity (AMI) are traditionally thought to be dispensable for immune control over the course of *Chlamydia* primary infection[23],[24]. Depletion of B cells using anti-IgM antibody showed minimum effect on resolution of *C. muridarum* infection[26]. B cell-deficient mice clear *C. muridarum* from the FRT with similar kinetics as WT controls[27]. Moreover, passive immune serum transfer into naïve mice fail to protect the hosts from *C. muridarum* intravaginal challenge[28]. While B cells seem to be dispensable at the FRT mucosa, our previous study reported an unexpected finding that *C. muridarum* causes disseminated infection in B cell-deficient (μMT) mice after intravaginal inoculation[29]. These mice exhibit delayed *Chlamydia*-specific CD4 T cell responses and develop ascites during the first a few weeks after *C. muridarum* FRT infection. We proposed several mutually non-exclusive mechanisms that could account for B cell-dependent control of disseminated bacterial infection: (1) antibody production; (2) B cell dependent antigen-presentation for prompt CD4 T cell responses in the local draining lymph nodes (DLNs); (3) The presence of B cells in the DLNs to maintain a proper architecture for effective CD4 T cell responses. In the current study, we sought to determine the detailed effector function of B cells in control of disseminated *Chlamydia*. We report that, antibody, but not B cell dependent antigen presentation, is essential for *C. muridarum* containment in the FRT.

Results

Generating mixed bone marrow chimeric mice

C. muridarum intravaginal inoculation typically induces a contained infection in the female reproductive tract (FRT). Mice deficient in B cells (μMT), however, experience a transient dissemination to distal tissues such as spleen and peritoneal cavity within 2 weeks post infection (Fig. 1A)[29]. This unexpected phenotype prompted us to further examine the

mechanism underlying this observation. To disrupt the function of B cells for antigen presentation independent from antibody production, we generated mixed bone marrow chimeras (BMCs) in which MHCII expression was specifically ablated in B cells ($MHCII^{B-/-}$) (Fig. 1B) [30],[31]. Loss of MHC class II expression on B cells was confirmed by staining splenocytes from reconstituted BMCs with CD19 and MHCII. As expected, $CD19^+$ B cells in $MHCII^{B-/-}$ BMCs stained negative for MHCII, whereas B cells in WT BMCs retained high levels of MHCII expression (Fig. 1C). $AID^{-/-}$ x $\mu S^{-/-}$ BMCs were generated in parallel to address the role of B cells in antibody production (Fig. 1B). Activation-induced deaminase (AID) is essential to initiate antibody class switching, and expression of secretory μ chain (μS) is required for antibody secretion[32],[33]. Thus, $AID^{-/-}$ x $\mu S^{/-}$ BMCs lack circulating antibodies while retain a polyclonal B cell population expressing functional BCRs and MHCII on their surface [34].

Chlamydia-specific Ab responses were partially diminished in $MHCII^{B-/-}$ BMCs

Lack of MHCII expression on B cells could potentially disrupt the cognate interaction between B cells and CD4 T cells, leading to defects in T cell-dependent antibody production. To assess whether Ab responses were affected by lack of MHCII-dependent T-B interactions during *Chlamydia* FRT infection, we measured antibody levels in $MHCII^{B-/-}$ BMCs by ELISA. Indeed, $MHCII^{B-/-}$ BMCs made significantly reduced serum Ig, as demonstrated by the universal reduction of all Ig isotypes measured (Fig. 2A–E). Specifically, high titers of IgG2b and IgG2c were only generated when B cells were expressing MHCII (Fig. 2B and 2C). In contrast, IgG3 responses that were typically induced by T-independent antigens were least affected in $MHCII^{B-/-}$ BMCs (Fig. 2D) [35]. These results suggest that *Chlamydia*-specific Ab production only partially relies on MHCII expression on B cells. Of note, although Ab responses were not completely abrogated in $MHCII^{B-/-}$ BMCs, neutralization activity of these Abs were not detected (Fig. 2F).

B-cell dependent antigen presentation is not critical for Chlamydia-specific CD4 T cell responses

We have previously reasoned that *Chlamydia* dissemination in B cell deficient mice is, at least in part, due to inefficient CD4 T cell priming in the local draining iliac lymph nodes (DLNs). Consequently, mucosal infections lack effective cell-mediated immune control and dissemination occurs to systemic tissues[29]. The question remains whether the diminished CD4 T cell priming in the DLNs was due to altered lymph node architecture and/or lack of B cell dependent antigen presentation in B cell deficient mice. To dissect the detailed mechanism, we assessed the magnitude of ag-specific CD4 T cell expansion in B cell BMCs using MHC class II tetramers (PmpG-1:I-A^b) specific for an endogenous *C. muridarum* epitope PmpG-1[29]. As expected, μMT BMCs exhibited reduced *Chlamydia*-specific CD4 T cell response in the DLNs at day 14 after intravaginal infection. In contrast, Ag-specific CD4 T cell numbers in $AID^{-/-}$ x $\mu S^{-/-}$ and $MHCII^{B-/-}$ BMCs were comparable to WT BMCs (Fig. 3A), indicating that both antibody and B cell dependent antigen presentation are dispensable for local CD4 T cell priming. Consistent with our previous observations, bacterial dissemination provoked robust systemic CD4 T cell responses in μMT and $AID^{-/-}$ x $\mu S^{-/-}$ BMCs, as demonstrated by the large increase of Ag-specific CD4 T cells in the spleens (Fig. 3B and 3C). A mild increase of *Chlamydia*-specific CD4 T cells was observed

in MHCII$^{B-/-}$ BMCs, while the mechanism remains unclear. Taken together, these results indicate that initial CD4 T cell expansion in response to *Chlamydia* intravaginal infection does not require antigen presentation by B cells or antibody. Therefore, it is likely that maintaining an intact lymphoid structure by B cells is crucial for efficient CD4 T cell responses.

Systemic dissemination of Chlamydia in the absence of antibodies

We next examined *Chlamydia* dissemination of various B cell BMCs. Consistent with our previous findings, high numbers of *C. muridarum* inclusion forming unites (IFUs) were detected in the peritoneal cavity of μMT BMCs at day 14 after *C. muridarum* intravaginal infection (Fig. 4A)[29]. Likewise, *Chlamydia* dissemination were also detected in AID$^{-/-}$ x μS$^{-/-}$ BMCs, as demonstrated by severe ascites and high *Chlamydia* IFUs in the peritoneal cavity (Fig. 4A). Disseminated infections in μMT and AID$^{-/-}$ x μS$^{-/-}$ BMCs were accompanied by increased F4/80^{+}Gr-1^{lo} macrophage infiltrates into the peritoneal cavity of these mice, as revealed by the analysis of ascites fluid from these mice (Fig. 4C and 4D). A mild increase of F4/80^{-}Gr-1^{hi} neutrophils was also observed in AID$^{-/-}$ x μS$^{-/-}$ BMCs (Fig. 4E). In contrast, no live *Chlamydia* or elevated macrophage/neutrophil infiltrates were detected in the intraperitoneal lavage of either WT or MHCII$^{B-/-}$ BMCs (Fig. 4A–E). In addition, bacterial burdens in the FRT as measured by vaginal swabs were similar among all groups (Fig. 4B). These results demonstrate that antibody, but not B cell-dependent antigen presentation, is essential for *Chlamydia* containment at the FRT mucosa.

Passive immune serum transfer rescues Chlamydia dissemination in B cell-deficient mice

To functionally complement the loss of antibody in B cell deficient mice, we next conducted passive serum transfer experiments to determine whether antibody is sufficient to rescue the *Chlamydia* dissemination phenotype in B cell-deficient mice. Immune convalescent serum from WT mice that had resolved a prior *C. muridarum* intravaginal infection were transferred intraperitoneally into μMT mice before intravaginally challenge with *C. muridarum*. Bacterial burdens were measured in systemic tissues including spleen, DLNs, mesenteric lymph nodes (MLNs) and peritoneal cavity. As shown in Fig. 5, disseminated bacteria in spleen, ILNs and peritoneal cavity were completely eradicated by immune serum transfer, but not by PBS or naïve serum treatment. Interestingly, live *Chlamydia* were found in mesenteric lymph nodes in μMT mice, and these bacteria were resistant to passive serum treatment. Consistent with previous reports[28], immune serum transfer had no effect on *Chlamydia* shedding from the FRT, as *C. muridarum* IFUs in the lower FRT were similar among all groups at the time of tissue harvest (Fig. 5E).

Discussion

The multifaceted roles B cells play in host defense against microbial pathogens have been studied extensively in various bacterial and viral infection models, but were rarely being interrogated for *Chlamydia*, the intracellular bacterium that accounts for the most prevalent bacterial STI worldwide. The lack of focus on B cells is largely due to the facts that both loss-of-function and gain-of-function studies in mouse models failed to establish a protective role for B cells during *Chlamydia* primary FRT infection[27],[28]. While revisiting the B

cell-deficient mice recently, we discovered that B cells are essential for *Chlamydia*-specific CD4 T cell priming in the DLNs and for preventing *Chlamydia* systemic dissemination[29]. These findings suggest that B cells play a more important role than previously appreciated, while the detailed mechanisms underlying the protective effect of B cells during *Chlamydia* primary infection remain to be elucidated.

Antigen sampling by APCs at the site of infection is the critical first step for launching the adaptive immune response. Amongst professional APCs that primes ag-specific CD4 T cells, dendritic cells (DCs) are the most potent APCs that activate naïve CD4 T cells[36]. B cells, in particular marginal zone B cells, has also been shown to participate Ag-presentation at early stage of T cell activation[37]. Our previous observation that *Chlamydia*-specific CD4 T cell was significantly reduced in the DLNs of B cell deficient mice implies that B cell-dependent Ag presentation may be essential for CD4 T cell priming[29]. Nevertheless, we show here that when MHCII expression is specifically ablated on B cells, clonal expansion of Ag-specific CD4 T cells remains intact. Therefore, the impaired early CD4 T cell responses in μMT mice may primarily attribute to the disrupted lymphoid architecture within the DLNs in the absence of B cells, and consequently inappropriate positioning of DCs and naïve CD4 T cells for effective interaction for CD4 T cell priming[38]. While our data does not exclude the possibility that B cells actively participate in antigen presentation during primary response, and B cells very likely serve as potent APCs during memory response[39], we speculate that submucosa DCs are the major APC population responsible for naïve CD4 T cell activation at the FRT mucosa[40]. These hypotheses will need to be tested experimentally.

The traditional dogma that intracellular bacteria are outside the reach of antibody-mediated immunity (AMI) has been challenged by emerging evidence showing protective antibody responses against intracellular pathogens including *Chlamydia*[28],[41]–[44]. Our data add to these findings and show definitively that antibody is essential for *Chlamydia* containment at the FRT mucosa. Antibody-deficient $AID^{-/-}$ x $\mu S^{-/-}$ BMCs suffer from significant bacteremia, an atypical phenotype following *Chlamydia* intravaginal infection, but closely mirrors the observation in B cell-deficient μMT mice. Moreover, antibody passive transfer fully complemented the defects of systemically disseminated infection in multiple tissues in μMT mice, including spleen, draining lymph nodes and peritoneal cavity. Of interest, bacterial shedding in the FRT were unaltered by immune serum treatment, indicating that *Chlamydia* infections in systemic vs mucosal tissues are likely controlled by distinct immune effector mechanisms. Numerous studies have demonstrated direct neutralization of *Chlamydia* EBs in in vitro tissue culture systems[45]–[47]. However, in vivo findings suggest that antibody does not elicit its effector function simply by neutralization. Consistent with this notion, while in vitro neutralization activity of Abs in $MHCII^{B-/-}$ BMCs was not evident (Fig. 2F), Ab response in these mice was clearly important for protection. It has been proposed that protective efficacy of antibody depends upon the local context and specific cell types or immune components present at systemic tissues and the pathogen-experienced FRT, but are absent from naïve FRT[48]. While such components for Ab-mediated protection remain elusive, recent studies by Morrison and colleagues shed light on the important roles of IFNγ and neutrophils in Ab-mediated protection against *Chlamydia* re-infections in the FRT[49],[50].

The immune convalescent serum we used in the serum transfer experiments preclude the possibility of evaluating the early Ab responses for bacterial containment, as high titer, high affinity antibodies are not present during the early stage of an infection. Our results, however, did suggest indirectly that early, T cell-independent Abs are responsible for bacterial containment. In MHCII$^{B-/-}$ BMCs, cognate interactions between B and T cells were diminished due to the lack of MHCII on B cells to interact with CD4 TCRs. Nevertheless, reduced Ab responses in these mice were sufficient to prevent bacterial systemic spread. This is consistent with other studies documenting early extra-follicular B cell response and its role in protection [48]–[50].

In addition to AMI, CMI plays a central role in host defense against *Chlamydia*. It is important to note that comparable numbers of Ag-specific CD4 T cells were detected in WT and antibody-deficient AID$^{-/-}$ x μS$^{-/-}$ BMCs, indicating that CMI was not sufficient to rescue the dissemination phenotype in the absence of AMI. Although this conclusion is seemingly contradictory with our previous argument that CD4 T cell response is essential for *Chlamydia* control in B cell-deficient mice, it is reasonable to speculate that CMI and AMI function synergistically yet independently at early stage of *Chlamydia* infection. Indeed, a more severe lethal disseminated infection was reported in mice lacking both arms of the adaptive immunity (*Rag1*$^{-/-}$), and mice lacking the major Th1 cytokine IFNγ also suffer from disseminated *Chlamydia* infection[51]–[53]. The non-redundant roles of CMI and AMI in *Chlamydia* dissemination were further elucidated by a recent study, in which Darville and colleagues showed that adoptive transfer of B cells successfully rescued the lethality of *Rag1*$^{-/-}$ mice, while bacterial burdens in lung tissues were only partially reduced[54]. Together, these findings contribute to the notion that mucosal surfaces are under stringent immune surveillance by collaborative efforts of CMI and AMI to exclude pathogen from systemic evasion.

In summary, we show in this study that antibody production, but not B cell-dependent antigen presentation to CD4 T cells, is essential for protecting the host from systemic *Chlamydia* dissemination. Future studies will be implemented to decipher the dynamics and mechanism of antibody-mediated protection in vivo and reveal other possible roles of B cells, such as cytokine production and regulatory functions in protective immunity against *Chlamydia*[55].

Materials and Methods

Mice

C57BL/6 (B6), μMT (B6.129S2-*Ighm*^{tm1Cgn}/J), MHCII$^{-/-}$ (B6.129S2-*H2*$^{dlAb1\text{-}Ea}$/J) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). AID$^{-/-}$ x μS$^{-/-}$ bone marrow cells were kindly provided by Dr. John Harty (University of Iowa). All mice used for experiments were 6–16 weeks old, unless otherwise noted. Mice were maintained under SPF conditions and all mouse experiments were approved by University of Arkansas for Medical Sciences and University of California Davis Institutional Animal Care and Use Committee (IACUC).

Generating bone marrow chimera

Recipient mice were given lethal irradiation at 1000 rad 4–6 h before reconstitution. Bone marrow from donor mice were isolated and five million cells were injected intravenously via the tail vein into the recipient mice. For mixed BMCs, μMT bone marrow was mixed with bone marrow from either MHCII$^{-/-}$ or WT (B6) at an 80:20 ratio. This ratio ensures that the majority (80%) of non-B cells in the BMCs express normal levels of MHCII. Host mice were kept on antibiotic treatment (polymyxin B 150 mg/L, neomycin sulfate 400 mg/L in drinking water) for 4 weeks, and analyzed 8 weeks after reconstitution.

Bacteria

Chlamydia muridarum strain Nigg II was purchased from ATCC (VR-123; Manassas, VA). Elementary bodies (EBs) were propagated in HeLa229 cells, purified by discontinuous density gradient centrifugation and stored at −80°C as previously describe[56],[57]. The stock was tested negative for mycoplasma contamination by both PCR-based mycoplasma detection kit (ATCC, Manassas, VA) and bacteria whole genome sequencing. A fresh aliquot was thawed and used for every infection experiment. The inclusion forming units (IFUs) of EB were determined by infection of HeLa229 cells and enumeration of *Chlamydia* inclusions stained positive with anti*Chlamydia* MOMP mAb (clone Mo33b, a generous gift from Dr. Harlan Caldwell, NIH).

Chlamydia infection and IFU enumeration

Mice were injected subcutaneously with 2.5 mg Depo-Provera (Greenstone, NJ) 5–7 days prior to intravaginal infection to ensure susceptibly at diestrus phase[58]. For infection, 1×10^5 *C. muridarum* EB in SPG buffer were deposited directly into the vaginal vault using a pipet tip. To enumerate bacterial shedding from the FRT, vaginal swabs were collected, disrupted with glass beads suspended in SPG buffer, serial dilutions were plated and IFUs enumerated on HeLa229 cells. To measure bacteria burdens in tissues, spleen, lymph nodes (LNs) were homogenized in SPG buffer, peritoneal cavity (PerC) was lavaged with SPG buffer. Tissue homogenate and lavage were shaked with glass beads. Samples were centrifuged at 500 g for 10 min, supernatants collected and serial dilutions plated on HeLa229 cells for IFU enumeration.

Chlamydia-specific serum Ab ELISA

Mice were bled retro-orbitally and serum was isolated. Heat-killed EBs (HKEBs) were prepared by heating EBs at 56°C for 30 min. High protein binding ELISA plates (Costar) were coated with 1×10^6 HKEB in 0.1 M carbonate/bicarbonate buffer (pH9.6) overnight at 4°C. Plates were blocked with PBS-T containing 0.1% non-fat milk for 1 hr at room temperature before serial dilutions of serum samples were added to the plates. *Chlamydia*-specific Abs were detected using HRP-based SBA Clonotyping System (Southern Biotech).

Ab neutralization assay

Antibody neutralization assay were conducted on Syrian hamster kidney (HaK) cells as previously described[59]. Briefly, mouse serum was collected via retro-orbital route and heated at 56°C for 30 min to inactivate complement activity. Serial dilutions of serum

samples were mixed with 1500 live EB SPG buffer containing 0.05% BSA at 37°C for 1 hr with shaking (450 rpm). Incubated samples were plated on HaK cells and IFUs enumerated as described above.

Tetramer staining and flow cytometry

Tetramer staining for *Chlamydia*-specific CD4 T cells was carried out as previously described [29]. Spleen and LNs were harvested from naïve or infected mice and single cell suspensions prepared in FACS buffer (PBS with 2% FCS) containing *Chlamydia* MHC class-II tetramers (PmpG-$1_{303–311}$:I-A^b) in Fc block (purified 2.4G2 mAb, 2% mouse serum, 2% rat serum) for 1 hr at 37°C in dark. Cells were washed and tetramer positive cells enriched via magnetic selection using EasySep PE Positive Selection Kit (Stemcell Technologies, Vancouver, BC). The enriched cells were surface stained with a panel of antibodies (listed below) and analyzed on an LSRFortessa flow cytometer (BD Biosciences, San Jose, CA). Antibodies used included B220 (RA3–6B2), CD4 (RM4–5), CD8 (53–6.7), CD11b (M1/70), CD19 (6D5), CD44 (IM7), CD90.2 (53–2.1), F4/80 (BM8), Gr-1 (RB6–8C5), MHCII (M5/114.15.2), Fixable Viability Dye (BioLegend, eBioscience, and BD Biosciences, San Diego, CA). Data were analyzed using FlowJo software (Tree Star, Ashland, OR). Flow cytometry procedures adhere to EJI recently published guidelines[60].

Passive serum transfer

Immune convalescent serum was isolated and pooled from B6 mice at >90 days after *C. muridarum* intravaginal infection. Five hundred microliters of immune serum, naïve serum or PBS was injected intraperitoneally into recipient mice on days −1, 0, 3 and 6 after infection[28].

Statistical analysis

Statistical analysis was performed by using an unpaired *t* test for normally distributed continuous variable comparisons and a Mann-Whitney U test for nonparametric comparisons (Prism; GraphPad Software, Inc.).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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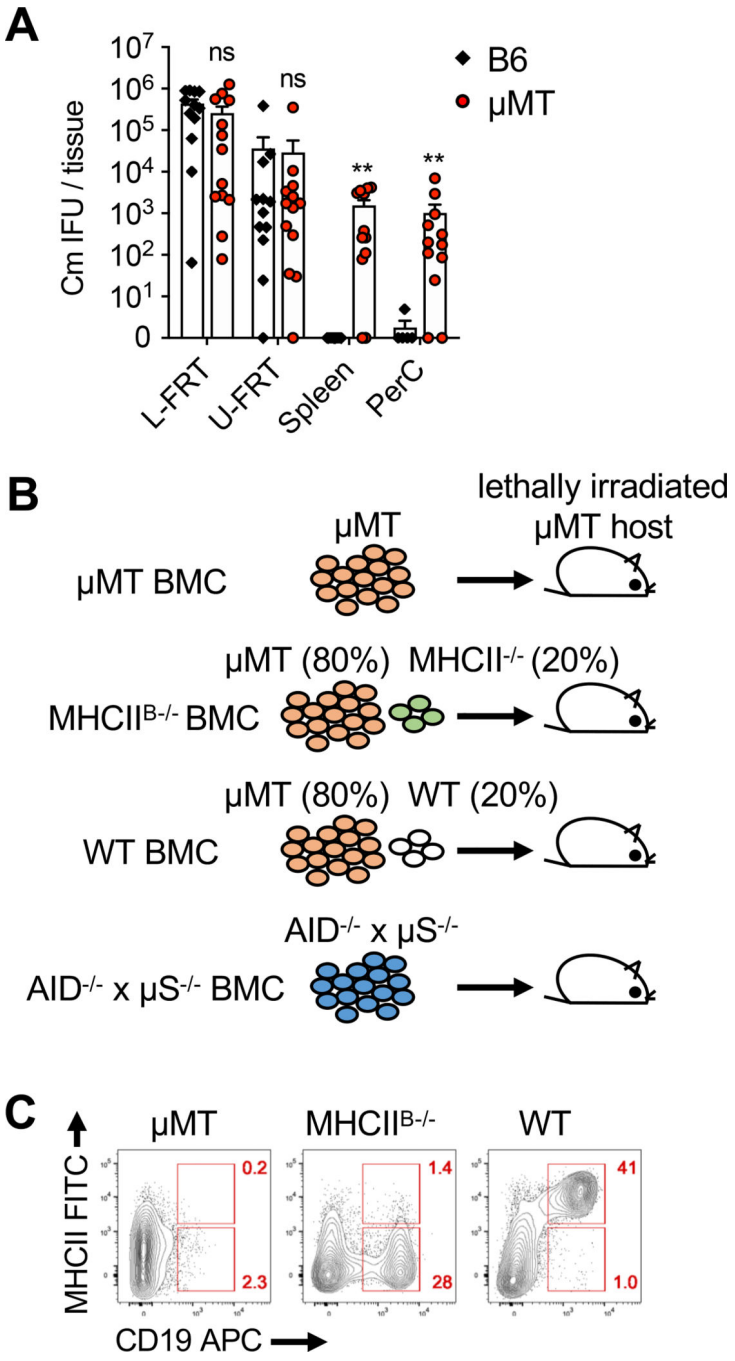

Fig. 1.

Bacterial dissemination in B cell deficient mice and generation of mixed bone marrow chimeras. (A) C57BL/6 (B6) and µMT mice were infected with 1×10^5 *C. muridarum* intravaginally. Bacterial burden in the lower (L) and upper (U) female reproductive tract (FRT), spleen and peritoneal cavity (PerC) 13–14 days post infection measured by enumerating IFUs on HeLa229 cells. Data shown are combined results of four independent experiments with a total of 12–13 mice per group. Error bars show mean bacterial counts ± SEM; **$p < 0.01$; ns, not significant as calculated by Mann-Whitney U test. (B) Cartoon

showing μMT bone marrow chimera (BMC) strategy. For $MHCII^{B-/-}$ BMCs, lethally irradiated μMT mice were reconstituted with μMT and $MHCII^{-/-}$ bone marrow cells mixed at an 80:20 ratio. Therefore, majority (80%) of non-B cells express normal levels of MHCII while none of the B cells express MHCII. (C) Flow cytometry plots showing expression of MHCII on $CD19^{+}$ B cell. Data shown are representative results of two independent experiments with 3–4 mice per group.

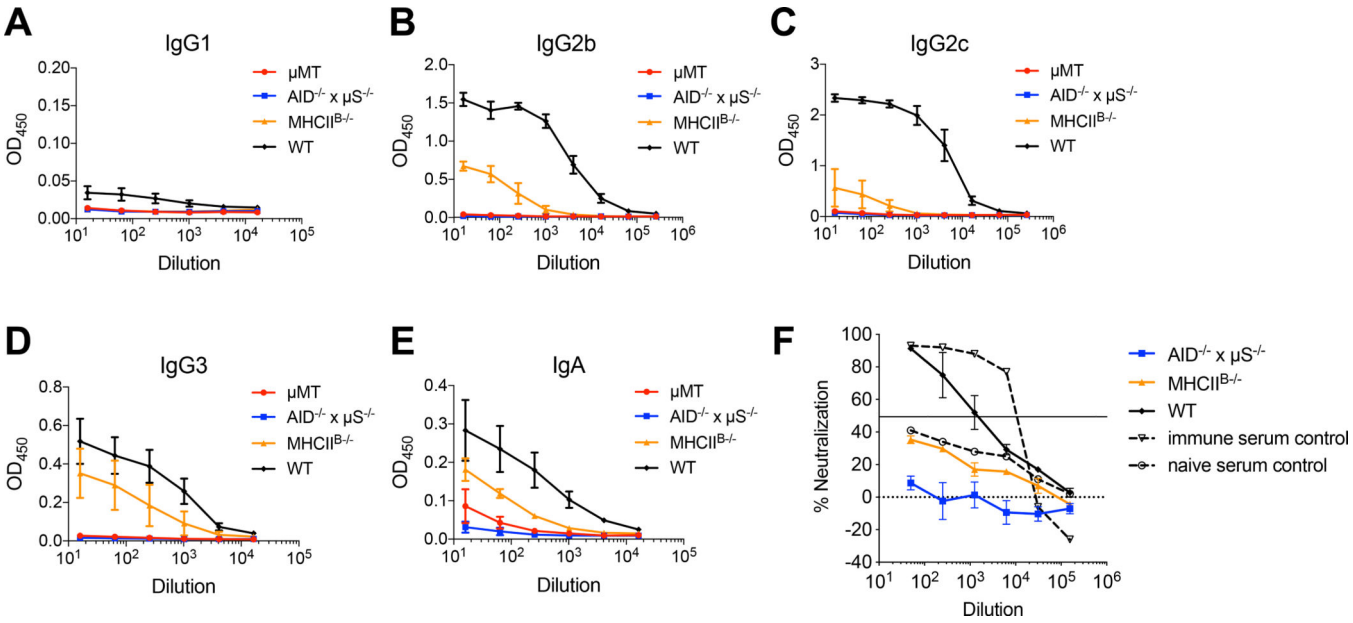

Fig. 2.

Serum Ab levels in BMCs 14 days after *C. muridarum* intravaginal infection.

WT, MHCII$^{B-/-}$, AID$^{-/-}$ x μS$^{-/-}$ and μMT BMCs were infected with 1×10^5 *C. muridarum* intravaginally. Fourteen days post infection, serum IgG1 (A), IgG2b (B), IgG2c (C), IgG3 (D) and IgA (E) were measured by antibody ELISA; neutralizing Ab levels (F) were measured by Ab neutralization assay. Data shown are representative results of two independent experiments with 3–4 mice per group. Error bars show mean bacterial counts ± SEM.

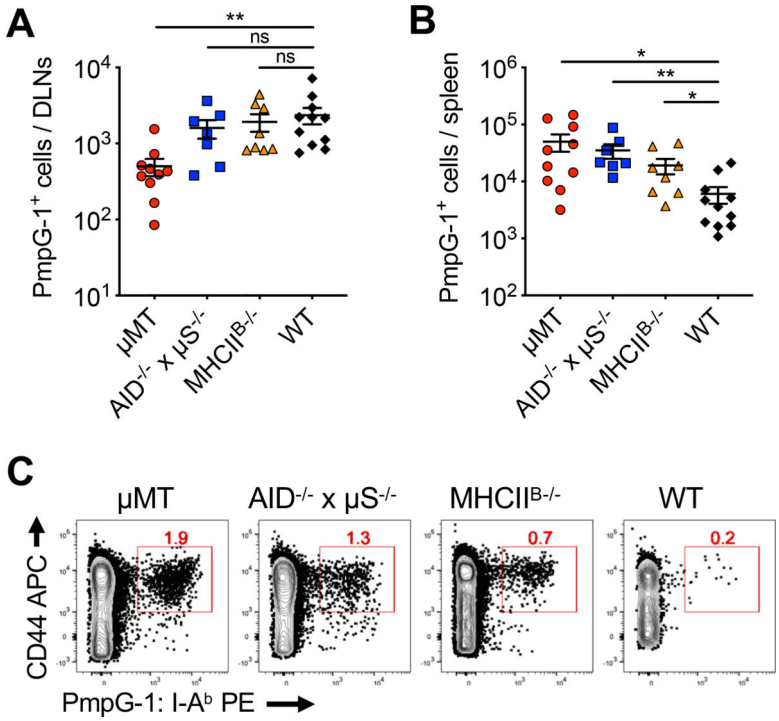

Fig. 3.

Chlamydia-specific CD4 T cell response is unaltered in the absence of B cell-dependent antigen presentation.

WT, MHCII$^{B-/-}$, AID$^{-/-}$ x μS$^{-/-}$ and μMT BMCs were infected with 1×10^5 C. muridarum intravaginally. Fourteen days post infection, *Chlamydia*-specific CD4 T cells from spleen and DLNs were analyzed by flow cytometry. (A-B) Total PmpG-1-specific CD4 T cells recovered from the draining lymph nodes (DLNs) (A) and spleens (B). (C) Flow cytometry plots showing representative PmpG-1-specific CD4 T cells from the spleen after tetramer staining and enrichment. All plots were pre-gated on CD11b$^-$F4/80$^-$B220$^-$CD3$^+$CD8$^-$CD4$^+$ cells. Total cell numbers of endogenous PmpG-1-specific CD4 T cells were calculated based on flow cytometry analysis. Data shown are combined results of three independent experiments with a total of 7–11 mice per group. Each data point represents an individual mouse. Error bars show mean ± SEM; *p < 0.05; **p < 0.01; ns, not significant as calculated by unpaired t-test.

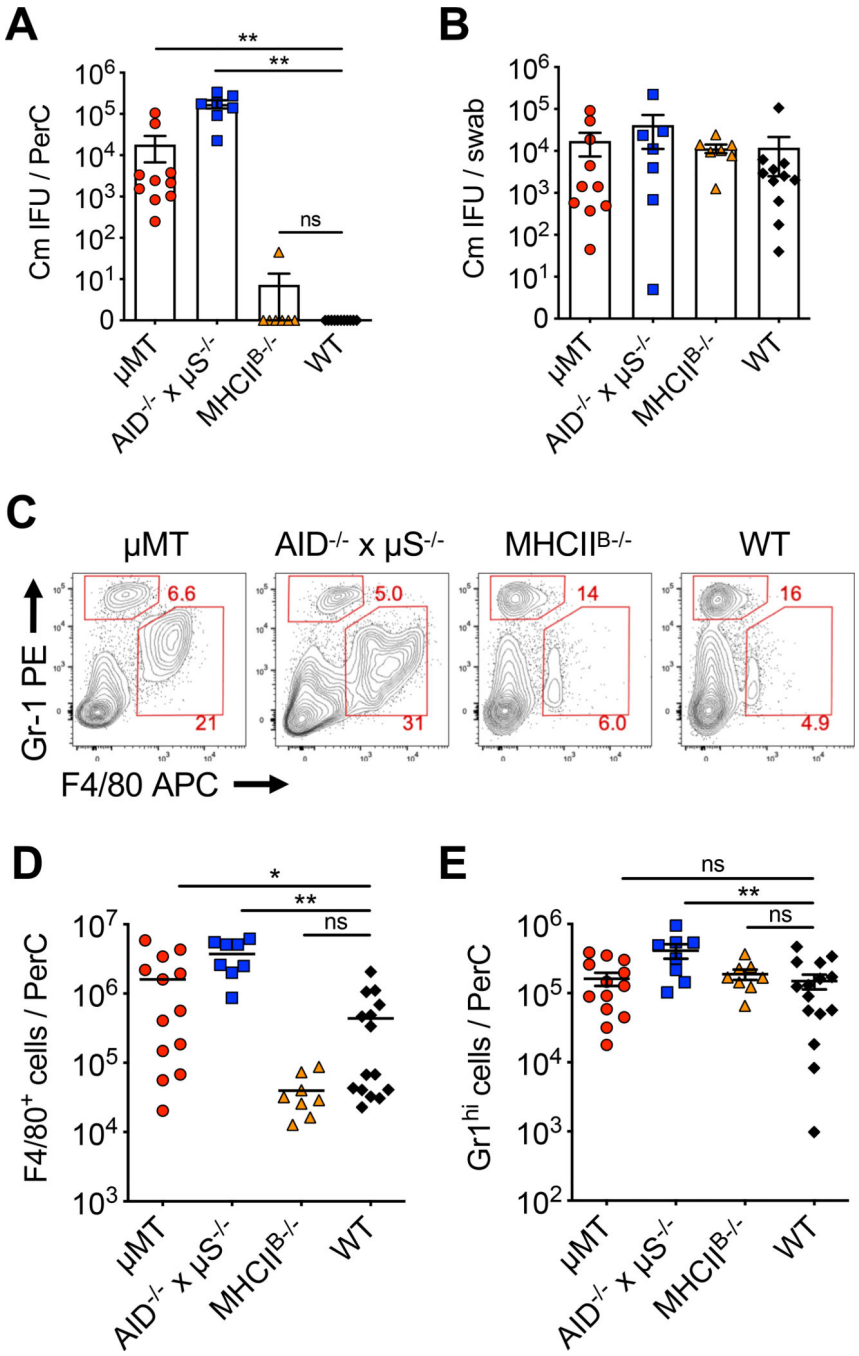

Fig. 4.

C. muridarum disseminates to the peritoneal cavity in AID$^{-/-}$ x μS$^{-/-}$ and μMT BMCs. WT, MHCII$^{B-/-}$, AID$^{-/-}$ x μS$^{-/-}$ and μMT BMCs were infected with 1×10^5 C. muridarum intravaginally. Fourteen days post infection, bacterial burden and cell infiltrates in the peritoneal cavity (PerC) were analyzed. (A-B) Bacteria burden in PerC (A) and the lower FRT (B) measured by enumerating IFUs on HeLa229 cells. Data shown are combined results of three independent experiments with a total of 7–11 mice per group. (C-E) Representative flow cytometry plots (C) and total cell counts of macrophages (F4/80$^+$Gr-1^{lo})

(D) and neutrophils ($F4/80^{-}Gr\text{-}1^{hi}$) (E) isolated from the peritoneal lavage. All plots were pre-gated on FSC/SSC to exclude doublets and dead cells/debris. Total cell numbers were calculated based on flow cytometry analysis. Data shown are combined results of four independent experiments with a total of 8–15 mice per group. Each data point represents an individual mouse. (A-E) Error bars show mean ± SEM; *$p < 0.05$; **$p < 0.01$; ns, not significant as calculated by Mann-Whitney U test for bacterial burden and unpaired t-test for cell numbers.

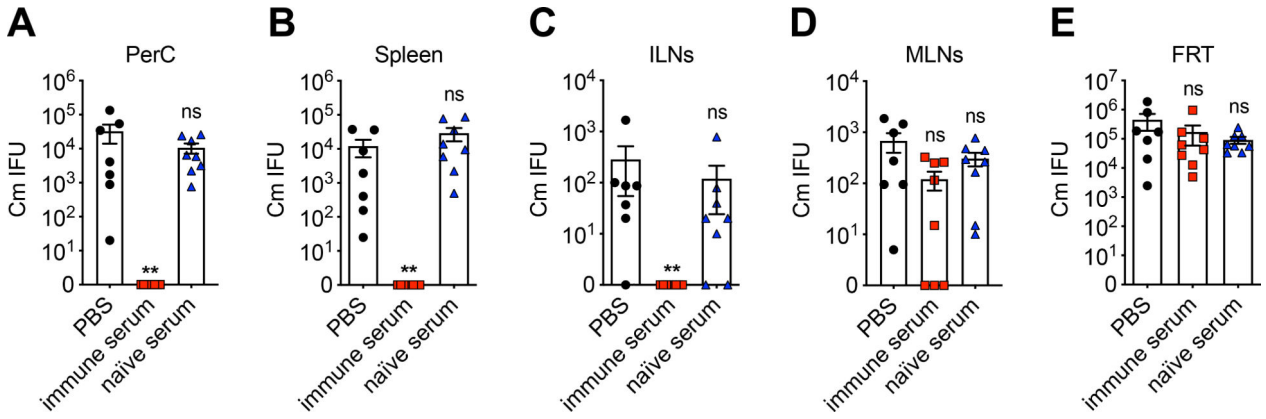

Fig. 5.

Passive immune serum transfers rescue disseminated *C. muridarum* from systemic tissues but not the FRT mucosa.

μMT mice were infected with 1×10^5 *C. muridarum* intravaginally. Five hundred microliters of immune serum, naïve serum or PBS was injected intraperitoneally into μMT mice on days −1, 0, 3 and 6 after infection. Bacterial burdens in peritoneal cavity (A), spleen (B), draining iliac lymph nodes (C), mesenteric lymph nodes (D) and lower FRT (E) on day 9 post infection as measured by enumerating IFUs on HeLa229 cells. Data shown are combined results of two independent experiments with a total of 7–8 mice per group. Each data point represents an individual mouse. Error bars show mean ± SEM; **p < 0.01; ns, not significant as calculated by Mann-Whitney U test.